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DNA TUMOR VIRUSES IN NON-PRIMATE SYSTEMS

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VIRUS ISOLATION/ STRUCTURE/ CHARACTERIZATION/ IDENTIFICATION

1. DISULFIDE BONDS PROTECT THE ENCAPSIDATED CHROMOSOMES OF SIMIAN VIRUS 40.

Ng SC, Bina M
Dept. Chemistry, Div. Biochemistry, Purdue Univ., West Lafayette, IN, 47906
FEBS Lett; 130(1):47-49 1981

To study the role of disulfide bridges in the protection of encapsidated simian virus 40 (SV40) DNA, the accessibility of viral DNA to micrococcal nuclease (MN) in the presence or absence of reducing agent dithiothreitol (DTT) was tested. Incubation of SV40 virions with increasing concentrations of MN in 5 milliM (mM) calcium chloride did not result in the cleavage of viral DNA. Incubation of SV40 virions with DTT and subsequent treatment with MN and calcium chloride (greater than 1 mM) resulted in digestion of viral DNA. Calcium chloride was only required for the activation of MN. Fractionation on the isokinetic sucrose gradients showed that the digested particles sedimented similarly to the intact virions. Since SV40 virions do not contain histones H1 or H5, it was

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concluded that specific types of interactions mediated by divalent ions, protein-protein and protein-DNA prevent the release of nucleosome cores generated by the action of MN on DTT-treated virions. (16 Refs)

2. STABLE ASSOCIATION OF VIRAL PROTEIN VP1 WITH SIMIAN VIRUS 40 DNA.

Brady JN, Lavialle CA, Radonovich MF, Salzman NP
Lab. Biology Viruses, Natl. Inst. Allergy and Infectious Diseases, Bethesda, MD, 20205
J Virol; 39(2):432-437 1981

Mild dissociation of simian virus 40 particles releases a 110S virion core nucleoprotein complex containing histones and the three viral proteins VP1, VP2, and VP3. The association of viral protein VP1 within this nucleoprotein complex is mediated at least partially through a strong interaction with the viral DNA. Treatment of the virion-derived 110S nucleoprotein complex with 0.25% Sarkosyl dissociated VP2, VP3, and histones, leaving a stable VP1-DNA complex. The VP1-DNA complex had a sedimentation value of 30S and a density of 1.460 g/cc. The calculated mol wt of the complex was 7.9 X 10(6), with an av of 100 VP1 molecules per DNA. Agarose gel electrophoresis of the VP1-DNA complex demonstrated that VP1 is associated not only with form I and form II simian virus 40 DNAs but also with form III simian virus 40 DNA generated by cleavage with EcoRI. (Author abstract) (15 Refs)

3. THE NATURE OF THE DNA ASSOCIATED WITH INCOMPLETE PARTICLES OF ADENOVIRUS TYPE 2.

Khittoo G, Weber JM
Dept. Biochemical Sciences, Princeton Univ., Princeton, NJ, 08544
J Gen Virol; 54(part2):343-355 1981

The nature of the DNA in incomplete particles (IP) synthesized by adenovirus Type 2 and the ts4 mutant which accumulates such particles were analysed by agarose gel electrophoresis, restriction endonuclease cleavage and blot hybridization techniques. IP DNA consisted of a heterogeneous population of subgenomic-size DNA (IPSD1) and smaller molecules ranging from about 1,000 base pairs to 200 base pairs (IPSD2). IPSD1 from ts4 was more heterogeneous than that from wild-type, but both contained sequences from all parts of the viral genome. IPSD2 contained heterogeneous cellular sequences and viral sequences from the left 4.4% of the genome. An endonuclease activity associated with IP and virions was capable of digesting viral or cellular DNA to IPSD2-like fragments suggesting a possible origin for these molecules. (Author abstract) (41 Refs)

4. THE DISTRIBUTION OF GUANINE - CYTOSINE PAIRS IN ADENOVIRUS DNAS.

Tikchonenko TI, Dubichev AG, Lyubchenko YuL, Kvitko NP, Chaplygina NM, Kalinina TI, Dreizin RS, Naroditsky BS
D. I. Ivanovsky Inst. Virology, U.S.S.R. Acad. Medical Sciences, Moscow, USSR
J Gen Virol; 54(part2):425-429 1981

The distribution of guanine-cytosine (GC) pairs in the DNA of the highly oncogen ic simian adenovirus Type 7 (SA7) and the non-oncogenic human adenovirus Type 6 (Ad6) has been studied by thermal denaturation and CsCl density-gradient centrifugation. The differential of the DNA thermal denaturation curves shows the presence of pronounced peaks which indicates uneven distribution of GC pairs along the DNA chains and the presence of regions with GC content from 30 to 74% in SA7 DNA and from 40 to 68% in Ad6 DNA. The DNA restriction fragments obtained by treatment with EcoRI, BamHI, SalI, BglII and HindIII were subjected to CsCl density-gradient centrifugation. GC content of the fragments ranged from 45 to 70% for SA7 DNA and from 43 to 61% for Ad6 DNA. The GC content of the extreme left-hand fragments, where the transforming gene(s) is located, was higher than the av for SA7 DNA and lower than the av for Ad6 DNA. The most GC-rich regions were localized in the center of the genome. The GC content of the right-hand part of both viral genomes was lower than the av. (Author abstract) (17 Refs)

5. MONOCLONAL ANTIBODIES TO TWO GLYCOPROTEINS OF HERPES SIMPLEX VIRUS TYPE 2.

Balachandran N, Harnish D, Killington RA, Bacchetti S, Rawls WE
Dept. Pathology, McMaster Univ., Hamilton, Ontario L8N 3Z5, Canada
J Virol; 39(2):438-446 1981

Monoclonal antibodies to herpes simplex virus Type 2 were found to precipitate different numbers of radiolabeled polypeptides from lysates of virus-infected cells. Antibodies directed against two viral glycoproteins were characterized. Antibodies from hybridoma 17alphaA2 precipitated a 60,000-mol-wt polypeptide which chased into a 66,000-and a 79,000-mol-wt polypeptide. All three polypeptides labeled in the presence of [3H]glucosamine and had similar tryptic digest maps. The 60,000-mol-wt polypeptide also chased into a 31,000-mol-wt species which did not label with [3H]glucosamine. Antibodies from hybridoma 17betaC2 precipitated a 50,000-mol-wt polypeptide which chased into a 56,000-and an 80,000-mol-wt polypeptide. These polypeptides also shared a similar tryptic digest map and labeled with [3H]glucosamine. Both monoclonal antibodies were herpes simples virus Type 2 specific. The viral proteins precipitated by 17alphaA2 antibodies had characteristics similar to those reported for glycoprotein E, whereas the proteins precipitated by 17betaC2 antibodies appeared to represent a glycoprotein not previously described. This glycoprotein should be tentatively designated glycoprotein F. (Author abstract) (23 Refs)

6. CHARACTERIZATION OF A PAPILLOMA VIRUS FROM THE EUROPEAN ELK (EEPV).

Moreno-Lopez J, Pettersson L, Dinter Z, Philipson L
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Virology; 112(2):589-595 1981

A novel papilloma virus (EEPV), morphologically resembling other members of the papovavirus group has been isolated from elk papillomas. The EEPV DNA is superhelical and has a mol wt of 5.4 x 10(6). Cleavage with the restriction endonucleases HaeIII, HindII, HindIII, and BglII suggests that the EEPV DNA is different from all bovine papilloma virus DNAs characterized so far. Hybridization between EEPV DNA and bovine papilloma virus type 1 or 2 DNA revealed no sequence homology under stringent hybridization conditions. (Author abstract) (11 Refs)

VIRUS ASSOCIATED CELL TRANSFORMATION AND HOST ALTERATION

7. CYCLIC AMP-DEPENDENT PROTEIN KINASES FROM BALB 3T3 CELLS AND OTHER 3T3 DERIVED LINES.

Wehner JM, Malkinson AM, Wiser MF, Sheppard JR
Dept. Genetics and Cell Biology, Univ. Minnesota, Minneapolis, MN, 55455
J Cell Physiol; 108(2):175-184 1981

Cyclic AMP-dependent protein kinase and 3H-cyclic AMP (3H-cAMP) binding activities were compared in normal Balb 3T3 cells and SV40, chemical, and spontaneous transformants of Balb 3T3. The photoaffinity analogue of cAMP, 8-N3 cAMP, was used to measure the amounts of R-I and R-II of protein kinase subunits in sonicates obtained from cells. Analysis of protein kinase activities showed no significant differences among normal and transformed cell types. The normal 3T3 cells prepared by homogenization showed lower soluble 3H-cAMP-binding activity than the transformed strains, but higher particulate activity. Apparently the method of cell disruption affected 3H-cAMP-binding activity in 3T3 cells, but not in transformed cells. Diethylaminoethanol chromatography showed that 3T3 cells contained only R-II isozymes. The transformed cells contained both R-I and R-II isozymes. Cells from rat kidney, chicken embryo, and human and calf endothelial tissue also contained both isozymes. The 8-N3 cAMP-binding confirmed that transformed cells have much more R-I than normal 3T3 cells. Evidently the 3T3 cells were derived from embryonic precursors of adipocytes and have retained a characteristic adipocyte isozyme pattern in culture. (37 Refs)

8. A MURINE STAGE-SPECIFIC EMBRYONIC ANTIGEN (SSEA-2) IS EXPRESSED ON SOME MURINE SV40-TRANSFORMED CELLS.

Shevinsky LH, Knowles BB, Howe C, Aden DP, Solter D
Wistar Inst. Anatomy and Biology, 36th St. at Spruce, Philadelphia, PA, 19104
J Immunol; 127(2):632-636 1981

A stage-specific embryonic antigen-2 (SSEA-2) found on murine preimplantation embryos is maximally expressed on 4- to 8-cell stage embryos and is present in decreasing amounts on morulae and blastocysts. This antigenic determinant is also expressed on murine teratocarcinoma cells, sperm, and some, but not all, SV40-transformed mouse cell lines. Analysis of solubilized immunoprecipitates by SDS-gel electrophoresis indicates that this cell surface molecule is not the 54,000 mol wt protein shared by teratocarcinoma and SV40-trans-formed cell lines. (Author abstract) (29 Refs)

9. CHARACTERIZATION OF A TEMPERATURE-SENSITIVE, DNA-POSITIVE, NONTRANSFORMING MUTANT OF SIMIAN VIRUS 40.

Cosman DJ, Tevethia MJ
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Virology; 112(2):605-624 1981

We have characterized an early mutant of SV40, tsA1642 which differs from other tsA mutants both in its location on the genome and in its phenotype. Marker rescue experiments position tsA1642 between 0.304 and 0.325 map units in the unique coding region for large T antigen. DNA sequencing within this region reveals a single nucleotide substitution at position 1782. Unlike other tsA mutants, the tsA1642 mutation does not result in the metabolic instability of large T antigen at nonpermissive temperature, nor does it lead to a defect in autoregulation of early gene expression. At nonpermissive temperature in the lytic cycle, tsA1642 accumulates viral DNA, T antigens, and late proteins at near wild-type levels, but produces only low levels of infectious virus. In addition to its defect in lytic growth, tsA1642 is markedly defective in transformation. It is unable to transform Brown Norwegian rat kidney (B/NRK) cells to anchorage-independent growth at 40.5. However, using the same assay, tsA1642 is nonconditionally defective in the transformation of a continuous line of C57B1/6 mouse embryo fibroblasts. TsA1642 generates transformed B/NRK cell lines some of which are temperature sensitive and some temperature resistant for the maintenance of the transformed phenotype. This mutant, therefore, appears to genetically separate, at least partially, some of the functions of large T antigen required for lytic growth from those required for transformation. (Author abstract) (60 Refs)

10. SIMIAN VIRUS 40 DNA SEQUENCES INTEGRATED WITHIN THE CHROMATIN OF SV40-TRANSFORMED CHINESE HAMSTER LUNG CELLS: I. TITRATION USING SINGLE-STRANDED DNA PROBES. II. PROGRAM OF REPLICATION WITHIN S PHASE AND THE EFFECTS OF SV40 T(TUMOR)-ANTIGEN.

Marchioni MA
Kansas State University, Manhattan, KS, 66502
Diss Abstr Int (B); 41(12):4356-B 1981

Nick-translated simian virus 40(32P) DNA was resolved into early (E)- and late (L)-strand nucleic acid sequences by hybridization with asymmetric SV40 cRNA. Both single-stranded DNA fractions contained less than 0.5 percent self-complementary sequences; and both included (32P) DNA-sequences that derive from all regions of the SV40 genome. These preparations have been used as probes to detect small amounts of viral DNA integrated into the chromosomes of Chinese hamster cells transformed by SV40. Under the conditions used for these titrations in soln (ie, 10-to 50-fold excess of radioactive probe), as lit tle as 1 pg of integrated SV40 DNA sequence has been assayed quantitatively. To determine when during S phase integrated viral DNA sequences in several tsA SV40-transformed Chinese hamster cell clones replicate, we pulse-labelled cultures with BrdUrd and subsequently collected mitotic cells during sequential time intervals. Our results support three conclusions: (1) specific genes with a mammalian DNA are programmed to replicate at precise x during S phase; (2) the programmed time for replication of a particular DNA sequence depends upon its location within the cellular genome; and (3) when the SV40 tsA gene product, T-antigen, was functional (ie, at permissive culture temperatures), all three tsA SV40-transformed cultures were reprogrammed to replicate integrated viral DNA sequences very early in S phase. The fractions of viral DNA replicated early in S phase appeared to correlate with each clone's content of functional T-antigen. (no Refs)

11. EXPRESSION OF SV40 T ANTIGEN IN FINITE LIFE-SPAN HYBRIDS OF NORMAL AND SV40-TRANSFORMED FIBROBLASTS.

Pereira-Smith OM, Smith JR
W. Alton Jones Science Center, Old Barn Road, Lake Placid, NY, 12946
Somatic Cell Genet; 7(4):411-421 1981

In order to determine the distribution of in vitro lifespans of hybrid cells resulting from the fusion of normal and Simian

virus 40 (SV40)-transformed cells, a biochemical selection system utilizing ouabain and HAT (hypoxanthine, aminopterin, and thymidine) was used. When normal human fibroblasts that had ceased proliferation were fused with SV40-transformed fibroblasts, cell proliferation was reinitiated in the resultant hybrids. This occurred even when the senescent normal parent cells had ceased replicating and were nonproliferative for 6 mo. In about 70% of the hybrids, the induced proliferative activity ceased within seven population doublings (PDs). In the remainder of the hybrid clones the proliferation was much more extensive (16-62 PDs). However, even these relatively long-lived hybrid clones eventually ceased proliferating. These results indicated that the processes that lead to in vitro senescence in normal cells can be reversed only temporarily by fusion with an SV40-transformed cell. UV irradiation appeared to impair the ability of SV40-transformed cells to reinitiate cell division. It was shown that the the hybrid clones which have limited division potential express viral T antigen. The hybrid clones seemed to contain viral DNA which was expressed, did not produce live virus, and continued to exhibit a limited lifespan. (24 Refs)

12. CLONING AND CHARACTERIZATION OF THE INTEGRATED VIRAL DNA FROM THREE LINES OF SV40-TRANSFORMED MOUSE CELLS.

Clayton CE, Rigby PW
Cancer Res. Campaign, Eukaryotic Molecular Genetics Res. Group, Imperial Coll. Science and Technology, London SW7 2AZ, England
Cell; 25(2):547-559 1981

Recombinant DNA techniques have been used to isolate the integrated viral DNA, together with flanking cellular sequences, from three independently isolated lines of SV40-transformed BALB/c 3T3 cells. We have determined the structures of these cloned segments and their relationships to one another by restriction enzyme mapping, electron microscopic heteroduplex analysis and transfer hybridization experiments. Our data indicate that the structure of the integrated viral DNA is consistent with the idea that integration involves a tandemly repeated intermediate, that extensive amplification and rearrangement of viral and adjacent cellular sequences occurs after integration and that the majority of viral DNA insertions do not contain an intact early transcription unit. The altered templates presumably encode the truncated and super T antigens found in these cells. Either they are interrupted in the middle of the early region, in which case they generally retain the region in which tsA mutations map, or they contain tandem duplications of the tsA region. Further structural analysis will allow the prediction of the precise structures of these unusual T antigens, and the availability of the corresponding templates opens the way to an analysis of the functions of these proteins. (Author abstract) (52 Refs)

13. LOSS OF FUNCTIONAL LARGE T-ANTIGEN AND FREE VIRAL GENOMES FROM CELLS TRANSFORMED IN VITRO BY POLYOMA VIRUS AFTER PASSAGE IN VIVO AS TUMOR CELLS.

Lania L, Hayday A, Fried M
Istituto di Biologia Generale e Genetica, Univ. Naples, Naples, Italy
J Virol; 39(2):422-431 1981

We have analyzed the state, arrangement, and expression of polyoma viral DNA sequences in a number of in vitro-transformed Fischer rat cells before and after growth in vivo as tumor cells. When the in vitro lines used to induce the tumors contained only a single insert of viral sequences and did not produce either a full-size 100,000-dalton (100K) large T-antigen or free viral genomes, no differences in the above-mentioned properties were observed. By contrast, in vitro cell lines containing multiple inserts of viral sequences, a functional 100K large T-antigen, and free viral genome induced tumor cells which displayed a reduced number of inserts of viral sequences and which did not produce either a functional 100K large T-antigen or free viral genomes. All of the in vitro lines and their tumor cell derivatives expressed the polyoma virus 55K middle and 22K small T-antigen species. Possible mechanisms for the selection in vivo against cells containing a functional 100K large T-antigen and consequently free viral genomes are discussed. (Author abstract) (21 Refs)

14. TRANSFORMATION OF RAT CELLS BY AN ALTERED POLYOMA VIRUS GENOME EXPRESSING ONLY THE MIDDLE-T PROTEIN.

Treisman R, Novak U, Favaloro J, Kamen R
Transcription Lab., Imperial Cancer Res. Fund Lab., London WC2A 3PX, England
Nature; 292(5824):595-600 1981

A modified polyoma virus genome has been constructed which can encode the middle-T protein, but not the large-T or small-T proteins. This was achieved, starting with the full length viral DNA inserted into a plasmid vector, by replacing a small genomic restriction fragment spanning the middle-T intervening sequence with the equivalent fragment from a cloned partial cDNA copy of the middle-T protein mRNA. Transfection of the modified viral DNA into cultured rat cells efficiently induced the formation of transformed cell foci which gave rise to cell lines that grew as tumors after injection into Fisher rats. The only viral early-region antigen synthesized by the cell lines was the middle-T protein. Expression of the middle-T protein is therefore sufficient to establish and maintain a transformed state. The viral mRNA produced by two of the transformed cell lines was structurally indistinguishable from the normal middle-T mRNA found in productively infected cells, suggesting that RNA splicing is not an essential step in the biogenesis of this messenger. (Author abstract) (48 Refs)

15. CLONING OF A DEFECTIVE POLYOMA DNA MOLECULE WITH REARRANGEMENTS IN THE REGION CODING FOR MIDDLE AND LARGE T ANTIGENS.

Parent C, Bastin M
Departement de Microbiologie, Centre Hospitalier Universitaire, Sherbrooke, Quebec J1H 5N4, Canada
FEBS Lett; 130(2):269-271 1981

To define the sequences of polyoma virus (Py) that are essential for cell transformation, defective Py DNA molecules were cloned in pBR322. The recombinant plasmids were tested for their capacity to transform rat 3T3 cells in culture. DNA from the polyoma virus strain ts-P155 was digested with HindIII and the fragments ligated in the HindIII site of pBR322 with T4 DNA ligase. To screen for defective molecules, the recombinant plasmids were selectively digested, and examined by agarose gel electrophoresis and endonuclease mapping. The Py DNA sequence in fragment df55 was found to be interrupted from approx 88-24 units before the HpaII-4/8 junction and the HindII site in HpaII-6. This deletion left the sequence encoding the small T segment intact but removed approx 1,800 base pairs (bp) from the large T antigen gene and about 530 bp from the middle T antigen gene. An interesting feature was the loss of approx 300 bp from the C-terminal portion of the middle T antigen gene. Transfection of 3T3 cells with df55-

containing recombinant plasmids failed to transform cultured cells kept for periods up to 3-4 wk at 39 C. The inability of pHdf55 to transform was ascribed to the absence of a functional middle T antigen. While not excluded, the small T antigen alone was not thought capable of inducing expression of the transformed phenotype. (16 Refs)

16. ALTERATIONS TO CONTROLS OF CELLULAR DNA SYNTHESIS BY ADENOVIRUS INFECTION.

Braithwaite AW, Murray JD, Bellett AJ
Dept. Microbiology, John Curtin Sch. Medical Res., Australian Natl. Univ., Canberra, A.C.T., 2601, Australia
J Virol; 39(2):331-340 1981

Human adenovirus type 5 and temperature-sensitive mutants ts36, ts37, and ts125 induced cellular DNA synthesis in quiescent rodent cells at both permissive and nonpermissive temperatures. Cellular DNA synthesis induced by adenovirus type 5 or by serum required protein synthesis for both initiation and continuation, whereas viral DNA synthesis was not dependent upon continued protein synthesis once it was initiated. Both cellular and viral DNA replication was induced in adenovirus type 5-infected cells in the presence of dibutyryl cyclic AMP at concentrations which inhibited induction by serum, which suggested that some of the controls of DNA synthesis in serum-treated and virus-infected cells are different. After adenovirus infection of quiescent cells, there was a decrease in the number of cells with G1 DNA content and an increase in cells with G2 diploid and greater DNA contents. Thus, adenovirus type 5 induces a complete round of cellular DNA replication, but in some cells, it induces a second round without completion of a normal mitosis. These results suggest that adenovirus type 5 is able to alter cell growth cycle controls in a way which may be related to its ability to transform cells. (Author abstract) (32 Refs)

17. IDENTIFICATION OF POLYPEPTIDE COMPONENTS OF ADENOVIRUS TYPE 12 TUMOR ANTIGEN FROM PRODUCTIVELY AND ABORTIVELY INFECTED CELLS AND FROM TUMOR CELLS.

Ribeiro G, Vasconcelos-Costa J
Unit. Virology, Gulbenkian Inst. Science, Apartado 14, P-2781 Oeiras Codex, Portugal
Virology; 112(2):775-779 1981

Adenovirus type 12 (Ad12) tumor antigen (T antigen) induced in productively infected KB cells, abortively infected rabbit RK13 cells, and hamster tumor cells (THA cells) was studied by immunoprecipitation and polyacrylamide gel electrophoresis. [35S]Methionine-labeled cell extracts were precipitated with anti-T serum, obtained from hamsters bearing tumors induced by Ad12, and with anti-P serum, obtained from rabbits immunized against Ad12 early proteins induced in RK13 cells. Two polypeptides with 31,000 (31K) and 19K mol wt are precipitated from all cells. Polypeptides with 16K, 15K, 13K, and 10.5K mol wt are observed in extracts from KB and THA cells only. T antigen from tumor cells also contains 57K, 50K, 45K, and 34K polypeptides. Polypeptides with 39K, 28K, and 25K mol wt are synthesized in KB and RK13 cells, mainly when the cells are pretreated with cycloheximide, but are never seen in extracts from tumor cells. Anti-P serum precipitates the 39K, 31K, 28K, 25K, and 19K polypeptides and also a 60K polypep tide from KB and RK13 cells, which probably corresponds to the DNA-binding protein. (Author abstract) (21 Refs)

18. HERPESVIRUS-INDUCED ANTIGENS IN SQUAMOUS-CELL CARCINOMA IN SITU OF THE VULVA.

Kaufman RH, Dreesman GR, Burek J, Korhonen MO, Matson DO, Melnick JL, Powell KL, Purifoy DJ, Courtney RJ, Adam E
Dept. Obstetrics and Gynecology, Baylor Coll. Medicine, Houston, TX, 77030
N Engl J Med; 305(9):483-488 1981

Tissue and serum specimens from 19 patients (24-79 yr old) with squamous-cell carcinoma (SCC) in situ of the vulva (10 patients), severe dysplasia (1), condyloma acuminatum (4), and controls seen for periodic check-up (4), were evaluated for herpes simplex virus Type 2 (HSV2) DNA-binding proteins. Nine of 10 biopsy specimens from patients with SCC or severe dysplasia reacted strongly when stained with anti-ICPS34/35 (rabbit antiserum to HSV2-infected-cell-specific protein 34/35), and eight also reacted with anti-VP143 (rabbit antiserum to nonstructural HSV2 protein); five also reacted with anti-ICSP11/12 (rabbit antiserum to HSV2-infected-cell-specific protein 11/12). All the positive reactivity observed with anti-ICSP34/35, anti-ICSP11/12 and anti-VP143 was localized in the cytoplasm rather than the nuclei, as has been previously reported in infected cells. Whole-virion structural antigens were not observed, although serological evidence of previous HSV2 infection was found in patients tested for the presence of antibodies. Serum antibodies to HSV2 were noted in nine patients with SCC, including one with mild dysplasia, and two with normal tissue. The paraliel rise in the incidences of HSV2 infection and of vulvar SCC in situ and the demonstration of HSV2-specific proteins in this tissue suggest an association of HSV2 infection with this cancer. (17 Refs)

19. TYPE 2 HERPES SIMPLEX VIRUS AND VULVAR CARCINOMA IN SITU.

Schwartz PE, Naftolin F
Yale Univ. Sch. Medicine, New Haven, CT, 06510
N Engl J Med; 305(9):517-518 1981

The association between herpes simplex virus Type 2 (HSV2) infection and the development of vulvar squamous cell carcinoma in situ (CIS) is discussed. The incidences of HSV2 infection and CIS of the vulva have risen dramatically in recent yr. The malignant potential of vulvar CIS remains to be established. The question of whether HSV2 infection is etiologically involved in the malignant transformation of CIS cells or is merely an opportunistic infection must be answered. The oncogenic potential of HSV2 for vulvar neoplasms must be investigated. (9 Refs)

20. HIGH-FREQUENCY TRANSFER OF CLONED HERPES SIMPLEX VIRUS TYPE 1 SEQUENCES TO MAMMALIAN CELLS BY PROTOPLAST FUSION.

Sandri-Goldin RM, Goldin AL, Levine M, Glorioso JC
Dept. Human Genetics, Univ. Michigan Medical Sch., Ann Arbor, MI, 48109
Mol Cell Biol; 1(8):743-752 1981

The protoplast fusion technique of Schaffner was adapted to determine if cloned herpes simplex virus-1 (HSV-1) sequences could be transferred to mammalian cells and expressed at high frequency. Bacterial cell walls were digested with lysozyme to produce protoplasts which were fused to mammalian cells by treatment with polyethylene glycol. Protoplasts were labeled with fluorescein isothiocyanate before fusion so that the transfer could be monitored. Since more than 50% of the mammalian cells with fluorescent after fusion, the bacterial material

was transferred with high frequency. In situ hybridization demonstrated that transfer of plasmid pBR325 occurred at frequencies of 1-2%. Following fusion transfer of a chimeric plasmid consisting of the HSV-1 (strain KOS) EcoRI fragment F in pBR325, approx 5% of the mammalian cells contained some of the viral genomic sequences. After fusion with protoplasts carrying the chimeric plasmid pX1, one Ltk- cell in 300-500 was transformed to the TK+ phenotype. (53 Refs)

21. HERPESVIRUS SAIMIRI DNA IN TUMOR CELLS-DELETED SEQUENCES AND SEQUENCE REARRANGEMENTS.

Desrosiers RC
New England Regional Primate Res. Center, Harvard Medical Sch., Southborough, MA, 01772
J Virol; 39(2):497-509 1981

Herpesvirus saimiri DNA in continuous lymphoblastoid cell lines obtained from viral induced tumors in marmosets has been analyzed by gel electrophoresis of restricted DNA, Southern transfer to nitrocellulose filters, and hybridization to 32P-labeled viral DNA or DNA fragments. The viral DNA fragments EcoRI-G, -H, -D, and -I, KpnI-A, and BamHI-D and -E were not detected in Southern transfers of DNA from the nonproducing 1670 cell line. For each restriction endonuclease, a new fragment appeared, consistent with a 13.0-megadalton deletion of viral DNA sequences. This deletion encompassed 35 to 48 +-0.6 megadaltons from the left end of the unique DNA region. A sequence arrangement map is presented for the major population of H saimiri DNA sequences in the 1670 cell line. Although H saimiri DNA in the nonproducing 70N2 cell line can be distinguished from viral DNA in the 1670 cell line by several criteria, the same sequences were found to be deleted in the major population of viral DNA molecules. Unlike 1670 and 70N2 cells, restricted DNA from the virus-producing cell lines 77/5 and 1926 contained all of the DNA fragments present in the parental virion DNA. DNA from 1670, 70N2, and 77/5 cells contained additional viral DNA fragments that did not comigrate with any virion DNA fragments. Most of these unexplained fragments were confined to or highly enriched in partially purified circular or linear DNA fractions. DNA from tumor cells taken directly from a tumor-bearing animal contained viral DNA indistinguishable from the parental virion DNA by the assay conditions used. These results indicate that viral DNA sequence rearrangements can occur upon cultivation of tumor cells in vitro and that excision of DNA sequences from the viral genome may play a role in establishing the nonproducing state of some tumor cell lines. (Author abstract) (15 Refs)

22. INDUCTION OF SEVERAL NEW ANTIGENS, INCLUDING A T CELL-ASSOCIATED ANTIGEN, FOLLOWING CONVERSION OF THE EBV-NEGATIVE B CELL LINE RAMOS TO EHRB-RAMOS BY EPSTEIN-BARR VIRUS.

Rosenthal KS, Shuman H, Strominger JL
Sidney Farber Cancer Inst., Harvard Medical Sch., Boston, MA, 02115
J Immunol; 127(2):746-754 1981

Heteroantisera prepared against EHRB-RAMOS cells and then absorbed extensively with the syngeneic EBV-negative cell line RAMOS recognized a membrane antigen present on EHRB-RAMOS but not on RAMOS cells. The binding of the sera, anti-EHRB, was measured by several immunologic techniques that detect cell surface molecules, including cytotoxicity, immunofluorescence, and the 125I-staph protein A assay. Triton X-100 extracts of plasma membranes prepared from EHRB-RAMOS competed for the binding of anti-EHRB on EHRB-RAMOS, as measured by the staph protein A assay, whereas extracts of RAMOS membranes did not. The anti-EHRB sera also recognized the EHRA-RAMOS cell line, but binding of anti-EHRB to other EBV-converted RAMOS lines--African Burkitt lymphoma lines, newly established and well established lymphoblastoid cell lines, and EBV producer cell lines--was undetectable. The anti-EHRB sera recognized 4 T cell lines established from T cell leukemias, including CEM, HSB, YT4E, and MOLT 4. In addition, an anti-CEM serum, prepared in this laboratory, recognized the EHRB-RAMOS cells. Absorption of the serum with peripheral blood lymphocytes removed the activity of anti-EHRB to both the T cell lines and to EHRB-RAMOS, as detected by immunofluorescence using the fluorescence-activated cell sorter. The EHRB-RAMOS cells still retained B cell characteristics, since they expressed p29, 34, an antigen found on normal peripheral B cells, and did not rosette with sheep RBC. The anti-EHRB sera and anti-CEM sera were further characterized by radioimmunoprecipitation of 35S-methionine-labeled proteins of EHRB-RAMOS, RAMOS, YT4E, CEM, and HSB. Anti-EHRB recognized polypeptides of 16, 32, and 35 x 10(3) daltons on EHRB-RAMOS and 85 x 10(3) daltons on EHRB-RAMOS, CEM, HSB, and YT4E. Anti-CEM recognized polypeptides of 95 to 130 x 10(3) daltons expressed on the T cells and EHRB-RAMOS. These polypeptides were absent from or expressed to a much less extent on RAMOS. Thus conversion of RAMOS by EBV to the EHRB-RAMOS B cell line was associated with the appearance of several different antigens, some of which were also present on 4 T cell lines and on peripheral blood lymphocytes (as evidenced by absorption). (Author abstract) (42 Refs)

23. TRANSFORMATION OF RODENT CELLS BY RFV, THE HUMAN PAPOVAVIRUS WITH DUAL GENOME.

Pater A, Pater MM, Dougherty RM, Di Mayorca G
Dept. Microbiology, Coll. Medicine and Dentistry New Jersey, New Jersey Medical Sch., Newark, NJ, 07103
Virology; 113(1):86-94 1981

Hamster, rat, and guinea pig cells were transformed by a variant of BK virus, RFV, whose genome consists of two complementary defective molecules, one, R1, with a deletion corresponding to 20-30% of the coding region for capsid proteins VP2 and VP3 and the other, R2, with a deletion corresponding approx to 50% of the coding region for large T-antigen of BKV. Blot-hybridization of cellular DNA reveals that all three transformed cell types contain viral DNA integrated into high-mol-wt DNA. Moreover, all the cell lines contain the viral DNA species. We examined the genome organization of the virus used in these transformation experiments to confirm our previous observations on the dual genome state of RFV. We found a very similar but not identical map for the plaque-purified RFV DNA used in this study when compared to that for plaque-purified RFV DNA examined previously. (Author abstract) (27 Refs)

VIRUS REPLICATION AND EXPRESSION

24. ANALYSIS OF TRANSCRIPTIONAL REGULATORY SIGNALS OF THE HSV THYMIDINE KINASE GENE: IDENTIFICATION OF AN UPSTREAM CONTROL REGION.

McKnight SL, Gavis ER, Kingsbury R
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Cell; 25(2):385-398 1981

We have identified a transcriptional control region of the herpes simplex virus tk gene. This finding results from in vivo transcription assays of specific deletion mutants constructed in vitro. A region located between 40 and 100 nucleotides upstream from the putative transcription start site of the tk gene can promote transcription by RNA polymerase Form II in the complete absence of the mRNA-coding component of the gene. When the region is deleted enzymatically from a 5' direction, accurate transcriptional expression is reduced by a factor of 50. The small remaining level of accurate transcription is eliminated by deletion of sequences from 32 to 16 nucleotides upstream from the structural gene. It appears that the sequences between 16 and 32 nucleotides upstream from the 5' terminus of the tk gene are required to specify the exact start site of transcription. Control of both the efficiency of transcription and rough specification of the position of initiation, however, depends on sequences 40-100 nucleotides upstream from the tk structural gene. (Author abstract) (49 Refs)

25. REPLICATION OF SIMIAN VIRUS 40 DNA IN NORMAL HUMAN FIBROBLASTS AND IN FIBROBLASTS FROM XERODERMA PIGMENTOSUM.

Ozer HL, Slater ML, Dermody JJ, Mandel M
Dept. Biological Sciences, Hunter Coll., City Univ. New York, New York, NY, 1002 1
J Virol; 39(2):481-489 1981

Simian virus 40 infection of semipermissive human diploid fibroblasts (HF), at early passage in cell culture, was compared with that of permissive established monkey cell lines. Viral DNA can be readily detected at 24 to 48 hr postinfection at 37 C with a high multiplicity of infection, approaching 10% of that of monkey cells (TC7). The length of time necessary for replication of an av molecule of viral DNA was found to be indistinguishable in HF and TC7 cells. Strand elongation plus termination were assessed by following the accumulation of DNA I at 40 C from replicative intermediates of tsA30 prelabeled at 33 C, obviating isotope pool problems. Combined initiation and elongation of wild-type viral DNA was measured by density shift experiments involving a 5-bromodeoxyuridine chase of prelabeled [3H]thymidine-labeled viral DNA. Determination of accumulation of viral T and V antigens supports the conclusion that the most likely basis for the reduced virus yield in HF cells results from the inefficiency of an early stage in virus infection, before or during uncoating. Similar results were obtained in fibroblasts derived from patients with xeroderma pigmentosum, suggesting that enzymes of uv repair are not required in unirradiated simian virus 40 DNA synthesis. (Author abstract) (34 Refs)

26. SIMIAN VIRUS 40 MATURATION: CHROMATIN MODIFICATIONS INCREASE THE ACCESSIBILITY OF VIRAL DNA TO NUCLEASE AND RNA POLYMERASE.

Brady J, Radonovich M, Lavialle C, Salzman NP
Lab. Biology Viruses, Natl. Inst. Allergy and Infectious Diseases, Bethesda, MD, 20205
J Virol; 39(2):603-611 1981

The accessibility of extracellular and nuclear simian virus 40 (SV40-M and SV40-I, respectively) virion chromatin DNAs to micrococcal nuclease, DNase I, BglI, EcoRI, and RNA polymerase was examined. Our results support the following conclusions: (i) the intranucleosomal DNA of SV40-I chromatin, similar to the precursor 75S chromatin complex, is resistant to enzymatic activity; and (ii) SV40-M virion chromatin is modified in a manner which increases the accessibility of viral DNA to enzymes, and the distinction between nucleosomal DNA and linker DNA is absent. Micrococcal nuclease digestion of SV40-I virion chromatin gave a typical nucleosomal DNA ladder pattern with a repeat unit of 205 base pairs of DNA. SV40-I chromatin was sensitive to cleavage with endonuclease BglI, but not with EcoRI. When SV40-I virion chromatin was used as a template, the rate of incorporation of ribonucleoside triphosphates into RNA was 5% of that obtained with naked SV40 form I DNA. Micrococcal nuclease digestion of SV40-M virion chromatin resulted in submonomeric DNA fragments of approx 55 base pairs, but no larger repeating unit of DNA was observed. SV40-M virion chromatin was sensitive to cleavage with either BglI or EcoRI and was approx 20% more susceptible to dig estion with DNase I than was SV40-I virion chromatin. The transcriptional efficiency of the extracellular virion chromatin was almost equivalent to that of naked SV40 form I DNA and was 16-fold higher than the rate observed with nuclear virion chromatin. The increased transcriptional activity was dependent upon the presence of nonhistone viral protein VP1 or VP2 or both. (Author abstract) (48 Refs)

27. A HIGH-RESOLUTION ELECTRON MICROSCOPY STUDY OF NUCLEOSOMES FROM SIMIAN VIRUS 40 CHROMATIN.

Moyne G, Freeman R, Saragosti S, Yaniv M
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J Mol Biol; 149(4):735-744 1981

The structure of nucleosomes in simian virus 40 chromatin was studied by conventional dark-field and by scanning transmission electron microscopy. We show that the DNA is located in the periphery of ovoid particles with a long axis of 89 A and a small axis of 79 A. Each of these particles contains 163 base-pairs. This value, the intensity of the peripheral stain and the path of the DNA entering and leaving the particles are compatible with the presence of two full turns of DNA around the central core of the histones. (Author abstract) (38 Refs)

28. SV40 DEFECTIVES SELECTED DURING LOW MULTIPLICITY PASSAGE ON A172 HUMAN GLIOBLASTOMA CELLS.

Carroll D, Hansen JL, Maryon EB, O'Neill FJ
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Virology; 112(2):461-471 1981

The glioblastoma line, A172, is unusual among human cell lines in being permissive for SV40 infection, and differs from

many host cell types in allowing the accumulation of viral defectives on low multiplicity passage. We have characterized these A172-derived defectives and find that most are reiteration mutants, containing multiple copies of both the viral replication origin (including the BglI site at map position 0.659) and sequences from the opposite side of the standard genome (around the BamHI site at map position 0.143). This distinguishes them from defectives accumulated during high-multiplicity passage in monkey cells, which generally have only reiterated origins. When well characterized, monkey cell-derived defectives (ev-1101, d5, a3, a4) were passed in A172 cells, the characterized defectives were lost, and sometimes replaced by other defectives present in the same stock. Surprisingly, this was true even when the characterized defective carried sequences from the BamHI region of standard SV40. These findings are taken to support the notion that sequences in addition to the viral replication origin are positively selected during defective propagation, but that different requirements for such sequences are se t by monkey cells and A172 cells. (Author abstract) (31 Refs)

29. REPLICATION OF SV40 CHROMATIN IN EXTRACTS FROM EGGS OF XENOPUS LAEVIS.

Richter A, Otto B, Knippers R
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Nucleic Acids Res; 9(15):3793-3808 1981

Simian virus 40 (SV40) nucleoprotein complexes were prepared from lytically infected cells and used as primer-templates for DNA replication in protein extracts from Xenopus eggs. We found that nucleoprotein containing replicating SV40 DNA served as primer-template while nucleoprotein with non-replicating SV40 DNA was ineffective. In vitro DNA synthesis begins with short DNA fragments ('Okazaki fragments') which are, in later steps, joined to give unit length SV40 DNA strands, suggesting that in vivo initiated rounds of replication are completed in vitro in the Xenopus system. This conclusion is supported by a restriction enzyme analysis showing that in vitro DNA synthesis occurs in fragments distal to the SV40 origin of replication. Our studies indicate that SV40 DNA replication in Xenopus extracts can be used as an experimental system to study the biochemistry of replicative DNA chain elongation in vitro. (Author abstract) (32 Refs)

30. CHROMATIN ASSEMBLY. RELATIONSHIP OF CHROMATIN STRUCTURE TO DNA SEQUENCE DURING SIMIAN VIRUS 40 REPLICATION.

Tack LC, Wassarman PM, DePamphilis ML
Dept. Biological Chemistry, Harvard Medical Sch., Boston, MA, 02115
J Biol Chem; 256(16):8821-8828 1981

The accessibility of five specific DNA sequences to six different single site restriction endonucleases was evaluated in replicating and mature simian virus 40 chromosomes isolated by three different methods. Electron microscopic and gel electrophoretic analysis of the DNA digestion products demonstrated that DNA accessibility in chromatin was established within 400 base pairs of replication forks and remained essentially unchanged during production of mature chromosomes and their subsequent re-e ntry into the replication pool. Saturating amounts of each en/me reproducibly cut a fraction of the chromosomes, ranging from 13 to 49%. This is consistent with a nearly random phasing of chromatin structure. Examples in which all chromosomes were either cleaved or intact were never observed. Although variation in the accessibility of DNA sites near the origin of replication could be interpreted as preferred phasing in about 25% of the chromosomes, the finding that two isoschizomers, Hpa II and MsP I, did not cut chromosomes to the same extent precludes an unambiguous interpretation of the extents of cleavage of individual restriction enzymes. Since the extent of DNA cleavage observed at each restriction site was essentially indistinguishable in replicating as compared to mature chromosomes, the accessibility of DNA sequences near the o rigin is not obviously related to replication. Furthermore, the accessibility of DNA sites on one arm of a single replication fork was the same as the homologous sites on the other arm, consistent with a nearly random phasing of chromatin structure on both arms. This suggests that chromatin assembly occurs independently on the two sibling molecules of a single replicating chromosome. (Author abstract) (72 Refs)

31. SV40 GENE EXPRESSION IS MODULATED BY THE COOPERATIVE BINDING OF T ANTIGEN TO DNA.

Myers RM, Rio DC, Robbins AK, Tjian R
Dept. Biochemistry, Univ. California, Berkeley, CA, 94720
Cell; 25(2):373-384 1981

We analyzed the DNA binding properties of wild-type simian virus 40 large T antigen and found that the protein binds cooperatively to three tandem sites at a regulatory region of SV40 DNA. One consequence of this T antigen: DNA interaction is the specific repression of SV40 early RNA synthesis in vitro. We mapped a region of 85 base pairs that is necessary and sufficient to initiate early SV40 transcription in vitro. This promoter region lies directly adjacent to the third T antigen binding site but does not include the 'TATA' sequence. To determine how T antigen interacts with its binding sites to repress RNA synthesis, we analyzed transcription directed by a variety of wild-type, mutant and hybrid template DNAs. Our findings suggest that the cooperative binding of T antigen to its sites is directly responsible for inhibiting the initiation, rather than blocking elongation, of early RNA synthesis. A model is presented to explain the role of T antigen binding in the regulation of viral transcription and DNA replication during SV40 lytic infection. (Author abstract) (68 Refs)

32. ADENOVIRUS TYPE 2 EARLY PROTEINS: ASSIGNMENT OF THE EARLY REGION 1A PROTEINS SYNTHESIZED IN VIVO AND IN VITRO TO SPECIFIC MRNAS.

Smart JE, Lewis JB, Mathews MB, Harter ML, Anderson CW
Cold Spring Harbor Lab., Cold Spring Harbor, New York, NY, 11724
Virology; 112(2):703-713 1981

We have investigated the relationships among the major proteins encoded by early region 1A of Ad2 by comparative analysis of their [35S]methionine-labeled tryptic peptides using reverse phase high-performance liquid chromatography. In some instances the purified peptides were subjected to automatic sequential Edmund degradation in order to determine the position at which the methionine residues occur within the peptides. These peptides have then been located in the amino acid sequence predicted from the DNA sequence of the leftmost 4.5% of the viral genome. The data show that the 50,000, 46,000, and 42,000 mol wt E1A proteins (all of which focus at ca pH 6.0) obtained from Ad2-infected HeLa cells are derived from the 1.1 kb region 1A mRNA. Similarly, it is shown that two polypeptides, 58,000 and 48,000, result fr om cell-free translation of the 1.1 kb mRNA. The data are also consistent with the hypothesis that the in vivo 46,000, 42,000, and 38,000

mol wt E1A proteins (all of which focus at ca pH 5.9) are derived from the 0.9 kb region 1A mRNA. Cell-free translation of this mRNA yields two polypeptides, 54,000 and 42,000. (Author abstract) (25 Refs)

33. A NONESSENTIAL GLYCOPROTEIN IS CODED BY EARLY REGION E3 OF ADENOVIRUS TYPE 7.

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Virology; 112(2):780-784 1981

Ad7 was shown to induce polypeptides of 66,000 daltons (66K), 28K, and 16K during early stages of infection. The 28K could be labeled with [2-3H]mannose and it bound to concanavalin A-agarose, indicating that it is a glycoprotein. Neither the glycoprotein nor the 16K polypeptide were induced by a viable early region E3 deletion mutant or an E3 insertion mutant, indicating that the polypeptides are coded by early region E3, and that they are nonessential for growth of Ad7 on cultured KB cells. The Ad7 glycoprotein was compared to the Ad2 E3-coded glycoprotein in terms of size and oligosaccharide linkage. The Ad7 glycoprotein has an apparent mol wt of 28K versus 25K for the Ad2 glycoprotein, as estimated by SDS-PAGE. The Ad7 glycoprotein and the purified 25K Ad2 glycoprotein were digested with endo-beta-acetylglucosaminidase H (endo H) to determine the type of oligosaccharide linkages as well as the apparent mol wt of the polypeptide chains. Endo H reduced the apparent mol wt of the Ad7 and Ad2 glycopolypeptides from 28K and 25K, to 17.5K and 19K, respectively. This establishes that both proteins have Asn-linked oligosaccharides of the high-mannose type, and that the Ad7 polypeptide chain is slightly smaller than that of Ad2, 17.5K versus 19K. Thus, the E3s of both Ad7 (group B) and Ad2 (group C) encode glycoproteins that are similar in apparent mol wt, that have high-mannose oligosaccharides, and that are nonessential for virus growth on cultured KB cells. (Author abstract) (22 Refs)

34. AMPLIFICATION OF PAPOVAVIRUS DEFECTIVES DURING SERIAL LOW MULTIPLICITY INFECTIONS.

O'Neill FJ, Carroll D
Dept. Cellular, Viral and Molecular Biology, Univ. Utah Coll. Medicine, Veterans Admin. Medical Center, Salt Lake City, UT, 84148
Virology; 112(2):800-803 1981

Many human cell lines share with A172 glioblastoma cells the ability to support lytic growth of SV40 and to accumulate defectives on low multiplicity passage. The latter is also characteristic of quite a number of host cells for BKV; thus, the need for high multiplicity infection to accumulate defectives is not as stringent as previously envisioned. Very few lines share with A172 the retention of reiterated viral termination regions in the defectives. (Author abstract) (11 Refs)

35. CHARACTERISTICS OF CHROMATIN PREPARATIONS FROM HERPES SIMPLEX VIRUS INFECTED CELLS.

Hay RT, Hay J
Dept. Biological Chemistry, Harvard Medical Sch., Boston, MA
Biochim Biophys Acta; 655(1):71-81 1981

Chromatin isolated from herpes simplex virus Type 1-infected baby hamster kidney cells contains a number of tightly associated virus-induced polypeptides. A subset of these proteins bind to immobilized DNA in vitro (Vmw 175, 155, 130, 63, 43, 38/39). Virus-induced polypeptides extractable with acid from infected cell chromatin include Vmw 155, the major capsid protein of herpes simplex virus Type 1 virions, and Vmw 63 and 38/39 which are heterogeneous with respect to change and are phosphorylated. These chromatin preparations, in the presence of deoxynucleoside triphosphates and MgCl2 were capable of synthesizing viral and cell DNA in a reaction which was stimulated by the addition of ATP, riboNTPs and potassium acetate. In vitro synthesized viral DNA cosedimented with prelabeled parental DNA but the single-stranded product was smaller than parental DNA. Density labeling indicated that extensive synthesis was taking place and all BamHI fragments of viral DNA were represented by the DNA synthesized in vitro. (Author abstract) (30 Refs)

36. HERPES SIMPLEX VIRUS TYPE 1 HINDIII FRAGMENT L ENCODES SPLICED AND COMPLEMENTARY MRNA SPECIES.

Frink RJ, Anderson KP, Wagner EK
Dept. Molecular Biology and Biochemistry, Univ. California, Irvine, Irvine, CA, 92717
J Virol; 39(2):559-572 1981

We have used DNA bound to cellulose to isolate and translate in vitro herpes simplex virus Type 1 (HSV-1) mRNA's encoded by HindIII fragment L (mapping between 0.592 and 0.647), an 8,450-base-pair (8.45-kb) portion of the long unique region of the viral genome. Readily detectable, late mRNA's 2.7 and 1.9 kb in size encoding 69,000-and 58,000-dalton polypeptides, respectively, were isolated. A very minor late mRNA family composed of two colinear forms, one 2.6 kb and one 2.8 kb, was isolated and found to encode only an 85,000-dalton polypeptide. A major early mRNA, 1.8 kb in size encoding a 64,000-dalton polypeptide, was also isolated. High-resolution mapping of these mRNA's by using S1 nuclease and exonuclease VII digestion of hybrids between them and 5' and 3' end-labeled DNA fragments from the region indicated that the major early mRNA contained no detectable splices, and about half of its 3' end was complementary to the 3' region of the very minor 2.6-to 2.8-kb mRNA's encoded on the opposite strand. These mRNA's also contained no detectable splices. The major late 2.7-kb mRNA was found to be a family made up of members with no detectable splices and members with variable-length (100 to 300 bases) segments spliced out very near (ca 50 to 100 bases) the 5' end. (Author abstract) (65 Refs)

37. CONSTRUCTION OF A DOUBLE-JOINTED HERPES SIMPLEX VIRAL DNA MOLECULE: INVERTED REPEATS ARE REQUIRED FOR SEGMENT INVERSION, AND DIRECT REPEATS PROMOTE DELETIONS.

Smiley JR, Fong BS, Leung WC
Pathology Dept., McMaster Univ., 1200 Main St. W., Hamilton, Ontario L8N 3Z5, Canada
Virology; 113(1):345-362 1981

In order to study the mechanisms of herpes simplex virus (HSV) DNA segment inversion and packaging of viral DNA, a mutant 'double-jointed' virus was constructed by inserting an additional copy of the L-S junction into the viral thymidine kinase gene. This additional joint promotes the formation of all of the novel joints and termini which are predicted to arise if any region of viral DNA which is flanked by inverted repeats is capable of inverting independently. In addition to promoting the formation of new isomeric forms of complete viral DNA by recombination between sets of inverted repeats, the additional joint participates in recombination events with direct repeats, resulting in the formation of two half-genomes. These

two half-genomes are both packaged into virions, but only as dimers. This result suggests that the recombination events which result in segment inversion frequently occur prior to the formation of concatemeric forms of viral DNA. (Author abstract) (30 Refs)

38. STRUCTURE OF REPLICATING HERPES SIMPLEX VIRUS DNA.

Jongeneel CV, Bachenheimer SL
Dept. Bacteriology and Immunology, Univ. North Carolina, Chapel Hill, NC, 27514
J Virol; 39(2):656-660 1981

We have investigated the molecular anatomy of the herpes simplex virus replicative intermediates by cleavage with the restriction endonuclease BglII. We find that in populations of multiply infected cells, pulse-labeled replicating herpes simplex virus DNA contains at least two and probably all four sequence isomers. Also, it contains no detectable termini. In pulse-chase experiments, we show that endless replicative intermediates are the precursors to virion DNA and that maturation is a relatively slow process. The results are discussed in terms of their significance to possible models of herpes simplex virus DNA replication. (Author abstract) (25 Refs)

39. HERPES SIMPLEX VIRUS TYPE I DNA POLYMERASE. KINETIC PROPERTIES OF THE ASSOCIATED 3'-5' EXONUCLEASE ACTIVITY AND ITS ROLE IN ARAAMP INCORPORATION.

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Dept. Pharmacology and Medicine, Sch. Medicine, Univ. North Carolina at Chapel Hill, Chapel Hill, NC, 27514
J Biol Chem; 256(16):8525-8530 1981

An exonuclease activity copurified with herpes simplex virus type I (HSV-1) DNA polymerase through DNA-cellulose column chromatography and comigrated with DNA polymerase activity on nondenaturing gel electrophoresis at varied polyacrylamide concentrations. A gapped duplex DNA was the preferred substrate for this exonuclease activity since the hydrolytic activity on this type of DNA was much greater than the hydrolysis of either native or heat-denatured DNA. Using 3'-terminally labeled activated calf thymus DNA as substrate, the exonuclease activity was found to be activated by salt and spermidine in a manner identical with HSV-1 DNA polymerase. This activation was accompanied by increases in apparent Km and Vmax values of the activated DNA substrate. Phosphonoformic acid inhibited both DNA polymerase and exonuclease activities uncompetitively with respect to activated DNA and had a Ki of 2.4 uM at an ionic strength of 0.25 u. Of the nucleoside 5'-monophosphates tested only the purine ribonucleotides inhibited the exonuclease activity. The inhibition was noncompetitive with respect to DNA, and GMP was about twice as potent as AMP or IMP. 9-beta-D-arabinosyladenine 5'-monophosphate (araAMP) could be incorporated into DNA by HSV-1 DNA polymerase; however, 9-beta-D-arabinosyladenine 5'-triphosphate would not replace dATP in supporting in vitro HSV-1 DNA synthesis. AraAMP incorporated into primer termini caused a significant decrease in the rate of subsequent primer elongation. These 3'-terminal araAMP residues could be removed by the HSV-1 DNA polymerase-associated exonuclease activity in a manner dependent on GMP concentration. (Author abstract) (21 Refs)

40. STUDIES ON THE NATURE OF MICRO-TUBULAR STRUCTURES AS MARKERS OF HERPES SIMPLEX VIRUS TYPE 2.

Iwasaka T
Dept. Microbiology, Kyushu Univ. Faculty Medicine, Fukuoka 812, Japan
Fukuoka Igaku Zasshi; 72(5):249-270 1981

The nature of minute tubular structures (TSs) found in Herpes simplex virus type 2(HSV-2)-infected cells was studied by examining the multiplication of HSV-2 and the appearance of TSs in cultured cells (Vero cells derived from African green monkey kidney cells) and in the uterine cervical tissue in 4-wk-old female C3H/N mice. Effects of UV irradiation and metabolic disorder inducing-drugs on the production of TSs were studied also, as were the dynamics of TSs appearing in HSV-1 and HSV-2 mixed infections. TSs were first observed at hr 5 and reached a max at hr 9 after infection of Vero cells with HSV-2 under conditions of moi 7. Similar tendencies were observed in the experiment with the C3H/N mice except that the virus replication and appearance of the TSs was delayed. TSs were observed to accumulate in the nuclei of HSV-2 infected cells when the cells were treated with hydroxyurea, cytosine arabinoside, and phosphonoacetic acid or irradiated with UV light, but the accumulation of TSs in the infected-cell nuclei was reduced when the cells were treated with 2-deoxy-D-glucose or cytochalasin B. Mixed HSV-1 and HSV-2 infection of cells resulted in a reduction of TSs. On the basis of the results, it was thought possible that TSs were structural co mponents of HSV-2 viron envelopes or their precursors. (41 Refs) (In JPN)

41. EPSTEIN-BARR VIRUS CO-RECONSTITUTED WITH SENDAI VIRUS ENVELOPES INFECTS EPSTEIN-BARR VIRUS-RECEPTOR NEGATIVE CELLS.

Shapiro IM, Klein G, Volsky DJ
Dept. Tumor Biology, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden
Biochim Biophys Acta; 676(1):19-24 1981

Epstein-Barr virus (EBV) was co-reconstituted with Sendai virus envelopes. The reconstituted 'hybrid' virus could bind and penetrate into EBV-receptor negative cells. Using this approach, T-cell-derived human and mouse leukemia cells, human T-lymphocytes and mouse spleen cells were successfully infected as judged by the induction of EBV-determined antigens and stimulation of DNA synthesis. The T-cell-derived human leukemia line Molt-4, that can absorb EBV but without virus penetration, could be also infected by the reconstituted EBV. (Author abstract) (27 Refs)

42. EARLY DNA-BINDING POLYPEPTIDES OF EPSTEIN-BARR VIRUS.

Roubal J, Kallin B, Luka J, Klein G
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Virology; 113(1):285-292 1981

The kinetics of Epstein-Barr virus (EBV)-specific protein synthesis has been studied after induction of P3HR-1 cells with n-butyrate. The majority of the EBV polypeptides became detectable on SDS-polyacrylamide gel electrophoresis between 15 and 18 hr after addition of the drug. Overall viral protein synthesis was at max after about 24 hr. Some polypeptides appeared significantly earlier (152K, 55K, 51K) while others were synthesized during a limited period of the cycle only (65K). At least four of the polypeptides detected (152K, 134K,

55K, 51K) bound to DNA cellulose columns. The EBV specificity of the polypeptides was confirmed by immunoprecipitation and by two-dimensional gel analysis. The 152K polypeptide had a pI around 8 whereas the other polypeptides varied between basic and acidic pI. The DNA binding proteins isolated by DNA cellulose chromatography represent a subclass of the virus-specific early proteins that can be immunoprecipitated from the total cell extract. (Author abstract) (18 Refs)

43. EPSTEIN-BARR VIRUS POLYPEPTIDES: IDENTIFICATION OF EARLY PROTEINS AND THEIR SYNTHESIS AND GLYCOSYLATION.

Feighny RJ, Henry BE, Pagano JS

Cancer Res. Center, Univ. North Carolina at Chapel Hill Sch. Medicine, Chapel Hill, NC, 27514

J Virol; 39(2):551-655 1981

We have identified at least six early polypeptides induced by Epstein-Barr virus in cells or under conditions which are nonpermissive for Epstein-Barr virus DNA replication ranging in mol wt from 140,000 to 26,000. (Author abstract) (18 Refs)

44. PHOSPHORYLATION OF RIBOSOMAL PROTEINS IN HAMSTER FIBROBLASTS INFECTED WITH PSEUDORABIES VIRUS OR HERPES SIMPLEX VIRUS.

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J Virol; 39(2):359-366 1981

In BHK cells infected with pseudorabies virus, there was a substantial increase in the phosphorylation of ribosomal protein S6. This increase occurred between 2 and 4 hr after infection and persisted at least until 9 hr. We estimated that in mock-infected cells S6 contained, on an av, one phosphate group per protein chain, whereas in infected cells this rose to between four and five phosphate groups per protein chain. A second ribosomal protein, either S16 or S18, was also phosphorylated after infection. No increase in cyclic AMP was found at the time of phosphorylation. We also found an increased phosphorylation of S6 in herpes simplex virus-infected BHK cells. (Author abstract) (39 Refs)

VIRAL GENETICS

45. MAPPING OF ADDITIONAL TEMPERATURE-SENSITIVE MUTATIONS (1600 SERIES) ON THE GENOME OF SIMIAN VIRUS 40 BY MARKER RESCUE.

Tevethia MJ, Slippey AE, Cosman DJ

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Virology; 112(2):789-794 1981

Eight temperature-sensitive mutations induced by either hydroxylamine or nitrous acid were positioned on the physical map of SV40 by marker rescue procedures. The map location of each mutation tested corresponded to its phenotype and complementation group assignment. Factors which may contribute to the unequal distribution of mutations on the viral genome are discussed. (Author abstract) (16 Refs)

46. INDIRECT INDUCTION OF MUTAGENESIS OF INTACT PARVOVIRUS H-1 IN MAMMALIAN CELLS TREATED WITH UV LIGHT OR WITH UV-IRRADIATED H-1 OR SIMIAN VIRUS 40.

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Proc Natl Acad Sci USA; 78(7,part2):4480-4484 1981

The frequency of mutants among the descendants of intact parvovirus H-1 was increased when cells of human and rat origin were exposed to UV light prior to infection. An enhanced mutagenesis of intact H-1 could be induced to almost the same extent in unirradiated cells by preinfecting them with H-1 or simian virus 40 that had been irradiated with UV light. The expression of an enhanced mutagenesis of unirradiated H-1 after cell treatment with UV light or UV-irradiated virus was delayed, transient, and dose-dependent, and it was dependent on de novo protein synthesis. (Author abstract) (21 Refs)

47. CLONING OF THE MARMOSET HERPESVIRUS THYMIDINE KINASE GENE AND ANALYSES OF THE BOUNDARIES OF THE CODING REGION.

Otsuka H, Hazen M, Kit M, Qavi H, Kit S

Div. Biochemical Virology, Baylor Coll. Medicine, Houston, TX, 77030

Virology; 113(1):196-213 1981

In order to delimit the approximate boundaries of the marmoset herpesvirus (MarHV) thymidine kinase (TK) gene, HindIII and BamHI digests of MarHV DNA were cloned in plasmid pBR322. Several recombinant plasmids which transformed E coli K12 strain RR1 to ampicillin resistance were isolated. The MarHV DNA inserts in these plasmids accounted for about half of the MarHV genome. One of the plasmids, pMAR4, contained a 9.1-kbp fragment of MarHV DNA (HindIII-G), transformed LM(TK-) cells to TK+, and hybridized to the BamHI-I fragment of MarHV DNA, which had previously been shown to have TK-transforming activity. pMAR4 DNA had little or no homology to the 2-kbp PvuII fragment of HSV-1 DNA, which contains the HSV-1 TK gene. Cleavage with PvuII, SacI, SmaI, and KpnI inactivated the TK-transforming activity of pMAR4, but cleavage with HindIII, PstI, EcoRI, XhoI, XbaI, and BamHI did not. Deletion mutants pMAR401 and pMAR420, which lacked the 2.6-kbp KpnI and the 2.75-kbp EcoRI fragments, respectively, of pMAR4, lost transforming activity, whereas pMAR410, which lacked a 2.9-kbp XhoI fragment of pMAR4 did not. Recombinant plasmid pMAR430, which contained a 3-kbp PstI fragment of pMAR4, also transformed LM(TK-) cells to TK+. The results strongly suggest that the coding region of the MarHV TK gene was within a 2.4-kbp pMAR4 sequence extending from the PstI (0.33 kbp) to the EcoRI (2.7 kbp) cleavage sites. (Author abstract) (41 Refs)

IMMUNOLOGICAL STUDIES

48. PROTECTIVITY OF HERPES SIMPLEX VIRUS ANTIGENS: STUDIES IN MICE ON THE ADJUVANT EFFECT OF PICLC AND ON THE DEPENDENCE OF PROTECTION ON T CELL COMPETENCE.

Hilfenhaus J, Christ H, Kohler R, Moser H, Kirchner H, Levy HB
Res. Lab. Behringwerke AG, D-3550 Marburg, W. Germany
Med Microbiol Immunol (Berl); 169(4):225-235 1981

Immunocompetent C57/6J Bom nu/+ mice and their T-cell-deficient nu/nu littermates were equally susceptible to primary herpes simplex virus type 1 (HSV-1) infections. The immunocompetent mice were protected against HSV-1 or type 2 (HSV-2) infections by preimmunization (2 wk before infection) with heat-inactivated whole virus or HSV-1 envelope antigen (HSV-1 EAG). Heat-inactivated HSV-2 was equally protective against HSV-1 and HSV-2 infections. Heat-inactivated HSV-1 was less protective against HSV-2 infections than against HSV-1 infections. The low immunizing capacity of HSV-1 EAG was greatly enhanced by using polyinosinic:polycytidylic acid complex with poly-L-lysine in carboxymethyl cellulose (PICLC) as an adjuvant. PICLC alone had no protective effect against HSV-1 infection. The immunoincompetent nu/nu mice were not protected against HSV-1 infection by preimmunization with HSV-1 EAG, even in PICLC adjuvant. It is concluded that T-cell competence is required for the establishment of a protective immunity against HSV infection by HSV-1 EAG preimmunization. (30 Refs)

49. IMMUNOLOGIC EVALUATION OF LYMPHOEPITHELIOMA (MEETING ABSTRACT).

Galligioni E, Carbone A, Tirelli V, Veronesi A, Trovo MG, Magri MD, Tumolo S, Grigoletto E
Ospedale Civile, Pordenone, Italy
Tumori; 67(2,SupplA):171 1981

Lymphoepithelioma (undifferentiated carcinoma of the nasopharyngeal type) is characterized by close correlation with Epstein-Barr virus (EBV). The titer of direct antibodies against EBV-associated antigens was determined in 13 consecutive patients (pat), 7 men and 6 women with a median age of 46 yr (16-57) observed between January, 1975, and December, 1980, at the Department of Radiology and the Oncology Clinic of the Pordenone Civilian Hospital. The primary tumor was located in the nasopharynx in 11 pat and in the palatine tonsils in 2. One pat was in stage II, all the others were in stage IV because of invasion of the base of the skull (2 pat), because of fixed or bilateral lateral cervical lymph nodes (7 pat), or because of distant metastases (3 pat). The titer of anti-EBV antibodies, especially IgA directed against viral capsid antigen (VCA) and early antigen (EA) turned out to be significantly elevated in 8 pat (3 men and 5 women) all in stage IV and all with tumor localized in the nasopharynx. In 3 of them the diagnosis of lymphoepithelioma was based on lateral cervical lymph node biopsy while the primary tumor in the nasopharynx was only found later (on av 1 yr later). In EBV-positive pat the antibody titer remained high even after complete remission in response to chemo-radiotherapy, nor was any correlation found between antibody titer and the probability of response to treatment or survival time (median observation 25 mo, range 3-54). Serologic investigation of EBV-associated antigen thus represents a useful diagnostic complement in pat with nasopharyngeal carcinoma especially in cases difficult to define histologically and may take on decisive inportance in pat with lateral cervical lymph node metastases without any signs of the primary tumor. (no Refs) (In ITA)

50. REDUCED PRODUCTION OF ANTI-HERPES ZOSTER ANTIBODIES, SPONTANEOUS OR AFTER CLINICAL INFECTION IN ADULT CANCER PATIENTS (MEETING ABSTRACT).

Moschen ME, Cartei G, Fiorentino MV
Ospedale Regionale, Padua, Italy
Tumori; 67(2,SupplA):46 1981

Serum concentrations of antivaricella complement-fixing antibodies (AVCFA) were measured in 182 cancer patients (from 20 to 60 yr old) with no history of clinically detectable zoster or varicella-zoster infection, and in a longitudinal sense in 12 cases after clinical manifestations of infection. For comparison a larger population of various ages and from the same region, cancer-free, was also studied during the course of infection. 'Spontaneous' production of AVCFA was much lower in cancer patient s (dilution from 0 to 1/32, av log 1/5; in the controls from 0 to 1/1024, av log 1/9); production secondary to infection was also quite compromised compared to those without cancer (at times completely absent). This amounts to demonstration of specific immunosuppression in cancer patients. (no Refs) (In ITA)

OTHER RELATED STUDIES

51. INHIBITION OF SIMIAN VIRUS 40 GROWTH IN A KIDNEY CELL LINE BY AN ENDOGENOUS INHIBITOR OF RENAL CELL PROLIFERATION.

Campadelli-Fiume G, Sinibaldi P, Busi C, Mattioli A, Fiume L
Istituto di Microbiologia e Virologia, Univ. Bologna, Via S. Giacomo 12, 40126 Bologna, Italy
Ann Sclavo; 23(1):52-56 1981

Chalones are non-cytotoxic, tissue-specific, non-species-specific substances that inhibit growth apparently by hindering deoxyribonucleotide polymerization. The effect of an inhibitor of epithelial kidney cell proliferation (IKCP) on viral proliferation was tested. BSC1 cells, a line of epithelial-like cells originated from African green monkey kidney, were infected with simian virus 40 (SV40). After virus adsorption IKCP was added to the cultures. All IKCP preparations hindered BSC1 cell proliferation. Above a threshold dose a dose-response relationship was observed. The IKCP preparations inhibited SV40 replication at the same concentrations which affected cell growth and which were non-cytotoxic. Apparently a number of endogenous cell growth inhibitors exhibit antiviral activity. Since they are non-cytotoxic, they may have pharmacological implications. (18 Refs)

52. HERPES VIRUS INACTIVATION BY CHEMICAL CARCINOGENS: DIFFERENTIAL INACTIVATION OF HERPES SIMPLEX VIRUSES BY 4-NITROQUINOLINE 1-OXIDE AND RELATED COMPOUNDS.

Speelman D, Li JL, Ramanujam VM, Legator MS, Albrecht T
(c/o Albrecht) Dept. Microbiology, Univ. Texas Medical Branch, Galveston, TX, 77550
Environ Mutagen; 3(6):467-476 1981

The effect of 4-nitroquinoline 1-oxide (NQO) on several strains of herpes simplex type 1 (HSV-1) and type 2 (HSV-2) was determined. The relationship between chemical structure and virus inactivation was examined. Relative rates of inactivation were first determined with HSV-1 (strain KOS) and HSV-

2 (strain 333) over a range of NQO doses. Loss of virus infectivity occurred at a minimum dose of 25 ug/ml NQO and increased as the concentration approached 200 ug/ml. HSV-1 strain KOS appeared more sensitive to NQO inactivation than HSV-2 strain 333 at various doses and intervals. The differences in rates of inactivation of these strains of HSV-1 and HSV-2 suggested that HSV-1 strains, in general, might be more susceptible to NQO inactivation. The data obtained with HSV-1 strains KET, Patton, and 71-15 and HSV-2 strains 79-18, 198, and MS at NQO doses of 50 ug/ml were consistent with those observed for HSV-1 strain KOS and HSV-2 strain 333, although strain KOS proved to be the most sensitive of the HSV-1 strains tested while strain 333 was the least sensitive of the HSV-2 strains tested. The structural specificity of 4-nitroquinolines required for HSV-2 inactivation was examined with a related group of chemicals. Resultant varying reductions in HSV infectivity indicated the specificity required for virus inactivation. (35 Refs)

53. VARICELLA AND HERPES ZOSTER IN 83 CHILDREN TREATED FOR MALIGNANCIES.

Gruson S, Mouchnino G, Reinert P, Patte C
Service de Pediatrie, Hopital Intercommunal, 94000 Creteil, France
Arch Fr Pediatr; 38(5):337-343 1981

The clinical and biological aspects of herpes zoster (26 cases) and varicella (57 cases) in 83 children being treated for various solid tumors (37) or leukemias and lymphomas (46) are described. Clinical symptoms of varicella in these patients included more numerous and more prolonged skin lesions, consciousness difficulties (10/57), and abdominal disorders (9/57). Abdominal disorders were found in patients with a more severe form of varicella: the two patients with melena died, as did 3/4 of the patients with intense, unexplained abdominal pain. Intense pain was present in 6/26 patients with herpes zoster, but no menacing visceral symptoms were observed. Lymphopenia of less than 1,000/cubic mm was observed in 37/57 of the varicella patients, and in 15/26 of the herpes zoster patients. Hemostatic, bacteriologic, and immunologic studies of these patients are described. The treatment of the patients with complicated (24) and uncomplicated (33) varicella is described. In 6/24 of the patients with complicated varicella, death occurred. The treatment of the patients with herpes zoster is described. (24 Refs) (In FRE)

54. CONSTRUCTION AND ISOLATION OF A TRANSMISSIBLE RETROVIRUS CONTAINING THE SRC GENE OF HARVEY MURINE SARCOMA VIRUS AND THE THYMIDINE KINASE GENE OF HERPES SIMPLEX VIRUS TYPE I (MEETING ABSTRACT).

Wei CM, Gilson ML, Spear P, Scolnick EM
Laboratory of Viral Carcinogenesis, National Cancer Institute, Bethesda, MD
RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

The src gene of Harvey murine sarcoma virus has been mapped to the 5' third of the Ha-MuSV genome. We have constructed lambda recombinants containing both the Harvey murine sarcoma virus (Ha-MuSV) genome and the thymidine kinase gene (tk) of Herpes simplex virus type I (HSV-1) linked to each other. The tk gene placed in a position downstream from the long terminal repeat (LTR) and the src gene of Ha-MuSV. The DNAs from the lambda recombinants were used to transfect NIH3T3 mouse fibroblasts in order to obtain Ha-MuSV DNA induced foci of transformed cells. The transformed foci were superinfected with a helper-independent retrovirus, and from the superinfected foci new individual retro viruses were isolated. The new v iruses can induce focus fo mation on NIH3T3 cells and convert NIH3T3tk- cells into th tk+ phenotype by carrying into the tk- cells the HSV-1 t gene. From virus infected cells, we have isolated nonproduce foci on NIH3T3 cells and tk+ transformants on NIH3T3tk cells containing one such new viral genome coding for th dual properties. Hybridization analysis indicates that the re combinant transmissible virus contains both the Ha-MuSV sr sequence and the tk gene sequence in a single RNA species c approx 4.9 kilobases. We conclude that retroviruses can b used as true vectors for genes other than genes that lead to ce cogenesis, and that the Ha-MuSV-tk recombinant virus nov offers a unique retrovirus vector system in which the genetic of src can be studied using the tk gene rather than src as a se lective marker. (no Refs)

55. BURKITT'S LYMPHOMA (BL): CLINICAL EVALUATION OF 11 PATIENTS (PAT) (MEETING ABSTRACT).

Crivellari D, Tirelli U, Carbone A, Volpe R, Veronesi A, Galligior MG, Trovo MG, Tumolo E, Grigoletto E
Div. di Radioterapia e Oncologia Clinica, Ospedale Civile di Porden one, Pordenone, Italy
Tumori; 67(2,SupplA):50 1981

From January, 1975, to December, 1980, 11 patients (pat with Burkitt's lymphoma (BL) were observed in our depart ment. There were 9 males and 2 females. Median age was 3. yr (range 9-57). 11 pat were born and 10 were living within radius of less than 150 km from Pordenone, 5 in cities and 6 i the countryside (3 in the same village). Staging included th bone marrow (BM) and periphery in 10 pat, lymphography i 8 pat, spinal fluid tap in 4 pat, Epstein-Barr virus (EBV) stud ies in 5 pat, and lactate dehydrogenase (LDH) analysis in 1 pat. 8 pat were stage III (Ziegler, 1972), all because of intra abdominal localization, 2 were stage IV (1 because of BM in volvement and 1 because of CNS involvement), and 1 pat wa stage I because of laterocervical localization. 3 pat were EBV positive and LDH was elevated in 5. 5 pat were treated surgi cally (S) with radical intent (all had intra-abdominal localiza tions) followed by chemotherapy (CT), the other 6 were treat ed with CT alone. In addition, 2 pat underwent radiotherapy (RT) for the purpose of CNS prophylaxis. Objective respons was obtained in all patients (3 complete remissions and 8 par tial remissions) but of short duration (median 3 mo). LDH proved a useful marker for follow-up in all the pat. Media survival was 8 mo (range 4-25); just 1 pat is surviving at 8+ mo without evidence of disease. Median survival was not im proved with S+CT+/-RT. (no Refs) (In ITA)

56. CORONAVIRUS-ASSOCIATED ANTIBODIES IN NASOPHARYNGEAL CARCINOMA AND INFECTIOUS MONONUCLEOSIS.

Arnold W, Klein M, Wang JB, Schmidt WAK, Trampisch HJ
Dept. Otorhinolaryngology, Klinikum Grosshadern, Marchioninstrass 15, D-8000 Munich 70, W. Germany
Arch Otorhinolaryngol (NY); 232(2):165-177 1981

Particles in the cytoplasm of nasopharyngeal carcinom (NPC) cells were found which resembled coronaviruses. T determine the nature of these particles serum titer determina tions were performed in NPC patients, infectious mononucleo sis patients, and a control group. Epstein-Barr nuclear antige (EBNA) test were made on tumor cells from NPC tissues an cells from cultures infected with coronaviruses. For immunolo

gical identification of coronavirus, the human coronavirus strain 229E was cultured on human fetal lung fibroblast MRC-C cells. Comparison of antibody levels showed clearly higher levels of IgG in NPC patients than in controls. IgA was the same in either group. IgM titer was in the low range in NPC patients. Infectious mononucleosis patients showed similar tiers to the NPC patients. The EBNA test was positive in all NPC cells, negative in cell cultures infected with coronavirus. In immunofluorescence tests serum from NPC patients produced intense fluorescence when applied to coronavirus-infected cells. Known high-titer coronavirus sera did not produce a similar result in MRC cells not infected with coronavirus. Evidently coronaviruses are present in the cytoplasm of NPC tumor cells. A correlation between the RNA coronavirus and the Epstein-Barr virus suggests a possible interaction between them as a factor in NPC. (35 Refs)

57. EFFECT OF AMINONUCLEOSIDE ON TRANSCRIPTION, METHYLATION, AND MATURATION OF RIBOSOMAL RNA IN SV40-TRANSFORMED HUMAN LUNG FIBROBLASTS.

Tunkel AR, Studzinski GP
(c/o Studzinski) Dept. Pathology, CMDNJ-New Jersey Medical Sch., 100 Bergen St., Newark, NJ, 07103
J Cell Physiol; 108(2):239-249 1981

Aminonucleoside of puromycin (AMS) was used to study the events leading to the production of mature ribosomal RNA (rRNA). SV40-transformed human embryonic lung fibroblast AG2804 cells were treated with AMS. The production of mature RNA was measured by assaying the uptake of 3H-methylmethionine and 3H-uridine, by measuring the incorporation of 3H-CH3 and 14C-uridine monophosphate into isolated nucleoli, and by RNA analysis. Treatment of fibroblasts indicated a rapid and biphasic effect of AMS on pre-ribosomal RNA (pre-rRNA). AMS inhibited transcription of pre-rRNA within 1-2 hr of exposure, and the inhibition was fairly constant. Pretreatment with AMS prior to measurement did not increase the inhibition significantly. Measurement of transcription and ribose methylation of pre-rRNA suggested that ribose methylation of pre-RNA was reduced only because of a lack of substrate. AMS apparently had no direct effect on methylation. The addition of fresh serum to intact cell cultures up to 2 hr after exposure to AMS produced increased transcription of pre-rRNA, indicating that the inhibitory effect of AMS was established in about 2 hr. Evidently AMS directly inhibits the transcription of pre-rRNA, while methylation of pre-rRNA and maturation of rRNA are inhibited later and indirectly. (44 Refs)

58. SEROEPIDEMIOLOGICAL STUDY OF HERPESVIRUS TYPE 2 AND CARCINOMA OF THE CERVIX.

Ohwada N
Dept. Obstetrics and Gynecology, Sch. Medicine, Gunma Univ., Maebashi-shi, Guma Pref. 371, Japan
Kitakanto Igaku; 31(2):87-97 1981

Sera obtained from patients with preinvasive and invasive cervical cancer, and ovarian and corpus cancer as well as from healthy control women were tested for neutralizing antibodies to herpesvirus type 1 (HSV-1) and type 2 (HSV-2) in regard to a possible association between HSV-2 and cervical cancer. Antibody titers to each virus type were determined simultaneously by a microtiter technique. The percentage of women with cervical cancer who had anti-HSV-2 antibody was significantly higher than that of patients with corpus and ovarian cancer and control women; cervical cancer patients 81%, corpus and ovarian cancer patients 26%, and control women 20%. However, the percentage of women having anti-HSV-1 antibody in the study group was comparable to that in the control group; cervical cancer patients 37%, corpus and ovarian cancer patients 39%, and control women 28%. The mean titer of neutralizing activity to HSV-2, as given by log2 of the reciprocal of the final serum dilution that exhibited the neutralizing activity, in the cervical cancer group (2.41 +-1.22) was also significantly higher than that in the corpus and ovarian cancer group (0.87 +-0.89) and in the control women (0.83 +-0.97). This difference was independent of age. It has been noticed that in some patients with cervical cancer, especially in the stage of carcinoma in situ, the neutralizing antibody titers decreased significantly after surgical resection o the cancer tissues. These findings suggest a strong association between HSV-2 infection and the establishment of cervical cancer, although it is not clear whether the virus has a causative role in the development of cervical cancer or not. (36 Refs) (In JPN)

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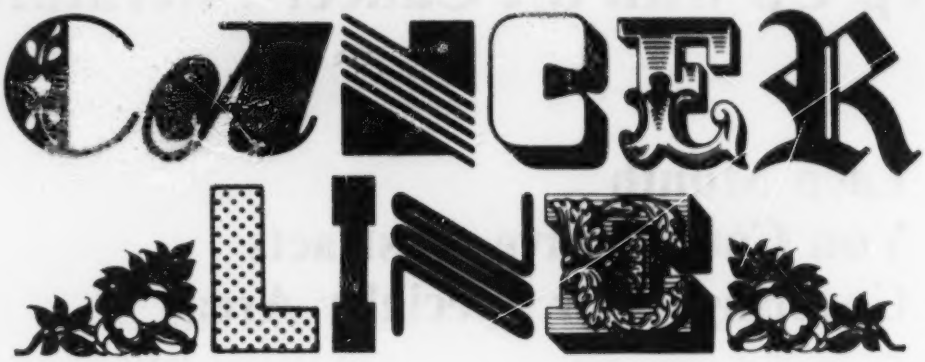

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VIRUS ISOLATION/ STRUCTURE/ CHARACTERIZATION/ IDENTIFICATION

1. PRODUCTION OF INFECTIOUS EPSTEIN-BARR VIRUS IN MOUSE LYMPHOCYTES.

Volsky DJ, Klein G, Volsky B, Shapiro IM
Dept. Pathology, Univ. Nebraska Medical Center, Omaha, NE, 68105
Nature; 293(5831):399-401 1981

Epstein-Barr virus (EBV) DNA replication and antigen expression were studied in CBA or A/Sn mouse lymphocytes rendered EBV-susceptible by virus receptor implantation. EBV-determined nuclear antigen (EBNA) and early and virus capsid antigens were demonstrated in receptor-implanted cells exposed to EBV strains P3HR-1 or B95-8. Although EBNA levels were low, viral DNA replication occurred in 0.3%-4.7% of the infected cells. Unlike human lymphocytes, the mouse lymphocytes could be infected by both EBV strains, and the infectibility was not limited to B lymphocytes. Moreover, infection was not followed by the outgrowth of permanently transformed lymphoid cells and suppression of the virus lytic cycle. Lytically infected mouse cells continued to release infec-

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tious virus that induced EBV antigens in target Raji and Lukes cells. (12 Refs)

2. RESTRICTION ENDONUCLEASE ANALYSIS OF THE GENOMES OF VIRULENT AND AVIRULENT MAREK'S DISEASE VIRUSES.

Hirai K, Ikuta K, Kato S
Dept. Molecular Biology, Tokai Univ. Sch. Medicine, Bohseidai, Isehara, Kanagawa 259-11, Japan
Microbiol Immunol; 25(7):671-681 1981

The comparative analysis of the fragments produced by cleavage of virulent BC-1 and avirulent C2(A) Marek's disease virus (MDV) DNAs with restriction endonucleases and preliminary results of the identification of the end fragments of these viral DNAs obtained with lambda 5'-exonuclease are reported. Restriction endonuclease studies indicated the possible presence of repeated sequences in MDV DNA. The presence of minor bands due to incomplete digestion of the DNA was unlikely. The presence of fragments in submolar amounts suggested the possibility of the existence of various populations of MDV DNA molecules with intramolecular arrangements of identical DNA sequences. Results of sedimentation of the DNAs of the BC-1 and C2(A) strains of MDV in a neutral glycol gradient are described. One end of MDV DNA appeared to be resistant to lambda exonuclease. Isopycnic centrifugation of the fragments of MDV obtained with restriction endonucleases indicated that MDV contains sequences of high guanine + cytosine content near one end of the molecule. (23 Refs)

3. BIOCHEMICAL ANALYSIS OF THE P30'S OF N-, B-, AND B--NB-TROPIC MURINE LEUKEMIA VIRUSES OF BALB/C ORIGIN.

Schindler J, Gautsch JW, Lerner RA, Hopkins N
Ortho Pharmaceutical Corp., Raritan, NJ, 08869
J Virol; 39(3):703-712 1981

Previous analysis of the virion proteins of an N- and a B-tropic Type C virus of BALB/c mice, of 16 N-tropic recombinants (XLPN viruses) between these viruses, and of eight NB-tropic viruses derived from the B-tropic virus suggested that among these closely related viruses N-, B-, or NB-tropism was associated with the electrophoretic mobility of p30 on sodium dodecyl sulfate-polyacrylamide gels, and thus that p30 might determine this phenotype. To obtain further evidence for the association of structural markers of p30 with N-, B-, or NB-tropism, we have analyzed the p30's of these same viruses by using two-dimensional tryptic peptide mapping and slab gel isoelectric focusing. The results of these analyses suggest that (i) a single peptide unique to the N-tropic virus p30 is present in the p30 of all N-tropic recombinants; (ii) a single peptide unique to the B virus p30 is not present in p30's of the N-tropic recombinants, and this peptide is also absent in p30's of NB-tropic viruses derived from the B-tropic virus; and (iii) p30's of NB-tropic viruses possess a new tryptic peptide not found in the p30 of their B-tropic virus progenitors, and this new peptide is not found in the p30 of the N-tropic virus of BALB/c or the XLPN viruses. These results are consistent with the possibility that p30 may determine the N-, B-, or NB-tropism of murine leukemia viruses. In addition, these studies indicate that some of the N-tropic recombinants have experienced recombination within the p30 gene. (Author abstract) (18 Refs)

VIRUS ASSOCIATED CELL TRANSFORMATION AND HOST ALTERATION

4. THE HUMAN BETA-GLOBIN GENE AND A FUNCTIONAL VIRAL THYMIDINE KINASE GENE IN DEVELOPING MICE.

Wagner EF, Stewart TA, Mintz B
(c/o Mintz), Inst. Cancer Res., Fox Chase Cancer Center, Philadelphia, PA, 19111
Proc Natl Acad Sci USA; 78(8):5016-5020 1981

Two foreign cloned genes - one encoding a tissue-specific protein and one encoding a constitutive enzyme - were introduced into mouse eggs by microinjection into a pronucleus shortly after fertilization. They were the adult human genomic beta-globin gene and the thymidine kinase (TK; ATP:thymidine 5'-phosphotransferase, EC 2.7.1.21) gene of herpes simplex virus (HSV), ligated in the pBR322 plasmid. Thirty-three developing mice were autopsied in late fetal life; all appeared normal. Blot hybridization tests revealed that the DNA of as many as five (15%) of the fetuses (from separate litters), and of their corresponding placentas, contained copies of the human beta-globin gene and of the HSV tk gene that had been retained and replicated without significant loss or rearrangement. The estimated total numbers of copies per cell were 3-50 for the donor globin gene and 3-20 for the donor tk. In some of the fetuses, these totals included some copies of mol wt higher than that of the intact sequence; the additional segments may have arisen through changes such as deletions or duplications. The foreign genes in the five positive fetuses appear to be present in high mol wt DNA. Assays capable of distinguishing between foreign and native TK indicated that at least one of the fetuses with the HSV tk gene had some TK enzyme of the HSV type and, therefore, at least one gene copy that was being accurately transcribed and translated to produce a functional protein, despite the absence of selective pressure. Thus, pure recombinant genes introduced into mice at the onset of their development can remain intact and be stably incorporated and even expressed. These experiments provide a practical basis for novel investigations of the developmental control of normal gene expression in vivo of the causes and possible cures of genetic diseases. (Author abstract) (35 Refs)

5. DEOXYRIBONUCLEASE I SENSITIVITY OF PLASMID GENOMES IN TERATOCARCINOMA-DERIVED ST EM AND DIFFERENTIATED CELLS.

Huebner K, Linennbach A, Weidner S, Glenn G, Croce CM
Wistar Inst. Anatomy and Biology, 36th St. at Spruce, Philadelphia, PA, 19104
Proc Natl Acad Sci USA; 78(8):5071-5075 1981

The DNase I (EC 3.1.21.1) sensitivities of the simian virus 40 (SV40) genome, the pBR322 genome, and the herpes simplex virus Type 1 thymidine kinase (HSV-1 tk) gene have been compared in teratocarcinoma-derived stem (12-1) and differentiated (12-1a) cell lines established by transfection of thymidine kinase (ATP: thymidine 5'-phosphotransferase, EC 2.7.1.21)-deficient F9 cells with DNA from a tripartite plasmid genome consisting of the pBR322 genome, the SV40 genome, and the HSV-1 tk gene. HSV-1 tk is present in both stem and differentiated cells; SV40 early proteins are present in differentiated cells but not in stem cells; the pBR322 genome is not expressed in either cell type. The SV40 and pBR322 genomes are more sensitive to DNase I digestion in stem cells than in differentiated cells, reflecting the DNase I-hypersensitivity of total stem-cell chromatin. The HSV-1 tk gene is the least sensitive to

DNase I digestion in both cell types. (Author abstract) (30 Refs)

6. DIFFERENCES IN THE DENSITY OF TOPOLOGICAL COILS OF DNA FROM NORMAL AND TRANSFORMED SYRIAN HAMSTER CELLS.

Glazer VM, Luchnik AN
M. V. Lomonosov State Univ., Moscow, USSR
Dokl Akad Nauk SSSR; 258(5):1227-1231 1981

The density of the topological coils of DNA was studied in normal Syrian hamster embryonal fibroblasts and in Simian virus 40 induced malignant tumors of Syrian hamsters. Cultured fibroblasts (10-12 passages) were subjected to sedimentation analysis in ethidium bromide-containing sucrose gradients. Sedimentation curves for normal fibroblasts showed shift to the left, while the cultured transformed cells were found to maintain a constant density of DNA coils. (13 Refs) (In RUS)

7. MONOCLONAL ANTIBODIES SPECIFIC FOR SIMIAN VIRUS 40 TUMOR ANTIGENS.

Harlow E, Crawford LV, Pim DC, Williamson NM
Dept. Molecular Virology, Imperial Cancer Res. Fund Lab., London WC2A 3PX, England
J Virol; 39(3):861-869 1981

Thirty hybridomas that secrete Ig against the simian virus 40 tumor antigens were isolated and cloned. Of these, 28 produced antibodies which bound to simian virus 40 large-T, and 2 produced antibodies which bound to the host 53,000-dalton protein. As in previous work, large-T antigen was found to have at least one determinant that it shared with small-t antigen and to have a minimum of six unique determinants. Several of the monoclonal antibodies from the L series hybridomas recognized determinants that were present on a subset of the large-T antigen from simian virus 40-transformed mouse cells. These monoclonal antibodies should be useful in studies of the structure and function of the simian virus 40 tumor ant igens. (Author abstract) (30 Refs)

8. TUBULIN ASSEMBLY SITES AND THE ORGANIZATION OF CYTOPLASMIC MICROTUBULES IN CULTURED MAMMALIAN CELLS.

Brinkley BR, Cox SM, Pepper DA, Wible L, Brenner SL, Pardue RL
Dept. Cell Biology, Baylor Coll. Medicine, Houston, TX, 77030
J Cell Biol; 90(3):554-562 1981

The number, distribution, and nucleating capacity of microtubule-organizing centers (MTOCs) has been investigated in a variety of cultured mammalian cells. Most interphase cells contain a single MTOC that is localized at the centrosome region and corresponds to the centriole and pericentriolar material. MTOCs, like centrioles, become duplicated during the S phase of the cell cycle and are equationally distributed to daughter cells in mitosis. Multiple MTOCs were rarely observed in cultured cells except in one cell line (neuroblastoma), which also displayed an equally large number of centrioles in the cytoplasm. The kinetics of microtubule assembly and the tubulin nucleating capacity of MTOCs are assayed by incubating tubulin-depleted, permeabilized 3T3 and simian virus 40-transformed 3T3 cells with phosphocellulose-purified 6S brain tubulin and microtubule assembly buffer. Initiation and assembly of 6S tubulin occurred in association with the cells' endogenous MTOCs, and the length, number, and distribution of microtubules generated about the organizing centers were regulated and cell specific. Our results are consistent with the notion that the specification of microtubule length, number, and spatial arrangement resides largely in the MTOCs and surrounding cytoplasm and not in the tubulin subunits. (Author abstract) (34 Refs)

9. CHARACTERIZATION OF A GENE ENCODING A 115 K SUPER T ANTIGEN EXPRESSED BY A SV40-TRANSFORMED RAT CELL LINE.

May E, Jeltsch JM, Gannon F
Institut de Recherches Scientifiques sur le Cancer, B.P. no 8, 94800 Villejuif, France
Nucleic Acids Res; 9(16):4111-4128 1981

It has been reported that SV40-transformed V 11 F 1 clone 1 subclone 7 rat cells (subclone 7) produce a super T antigen of 115,000 M. This super T antigen is entirely SV40 coded and is synthesized by translation of an elongated form of SV40 early mRNA. The results reported here show that there is only one independent insertion of viral DNA in the cellular genome of subclone 7 cells. When DNA from subclone 7 cells was cleaved with Bam HI endonuclease two distinct SV40 sequence containing fragments were generated with sizes of 5 Kb and 10 Kb, respectively. Two recombinant cosmids were constructed by insertion of the 5 Kb and 10 Kb fragments, respectively, into cosmid pHC 79. Using restriction map analysis and nucleotide sequencing, we showed that the 5 Kb fragment actually contained the complete sequence of a gene encoding super T antigen. As compared to the normal SV40 early gene, the sequence of super T gene showed the following rearrangements: (i) The segment between nucleotides 4116-3544 was duplicated in a direct order and (ii) these two copies of 573 nucleotide sequence were separated by a 93 nucleotide tract which was a nearly perfect inverted repeat of the segment located between nucleotides 4868 and 4776 (nucleotide numbering used here = Weissmann number + 17). (Author abstract) (30 Refs)

10. IMMUNOSELECTION OF SIMIAN VIRUS 40 LARGE T ANTIGEN MESSENGER RNAS FROM TRANSFORMED CELLS.

Oren M, Levine AJ
(c/o Levine) Dept. Microbiology, State Univ. New York Sch. Medicine, Stony Brook, NY, 11794
Virology; 113(2):790-793 1981

The messenger RNAs (m-RNAs) coding for the simian virus 40 (SV40) large tumor antigen were substantially purified by immunoprecipitation of polysome fractions from SV40-transformed cells. The level of SV40 T antigen m-RNA in the transformed cell used in this study was estimated at 0.05% of the total population of polyadenylated m-RNAs and a several-hundred-fold purification of this m-RNA was achieved by employing antibodies to SV40 large T antigen purified by either of two methods. The procedures described in this communication should be particularly useful for the immunoselection and purification of low-abundance m-RNAs or when the antigen is not available in large quantities. (Author abstract) (12 Refs)

11. FUNCTIONS OF T ANTIGENS OF SV40 AND POLYOMAVIRUS.

Hand R
McGill Cancer Centre, McGill Univ., 3655 Drummond, Montreal, Quebec, H3G 1Y6, Canada
Biochim Biophys Acta; 651(1):1-24 1981

Functions of the tumor or T antigens of SV40 and polyomavirus are reviewed. Topics discussed include genome organiza-

tion (SV40 genome, polyomavirus genome, and comparison of maps), proteins of the early regions (SV40 antigens, polyomavirus T antigens, and super T antigens), functions of T antigens (small t antigen, middle T antigen, and large T antigen), other immunoreactive T antigens (tumor-specific transplantation antigens, U antigen, and cellular proteins bound by antibodies against SV40 T antigens), large T antigen binding to DNA, viral DNA synthesis, cellular DNA synthesis (SV40 and polyomavirus), adenovirus helper function, regulation of viral transcription, integration of viral DNA, enzyme reactivities associated with T antigens (SV40 and polyomavirus), T antigens and transformation (SV40, polyomavirus, and A and N transformants), and conclusions. (196 Refs)

12. HUMAN FETAL BRAIN CELLS IN CULTURE. INCREASE IN GM2 GANGLIOSIDE AFTER SV-40 TRANSFORMATION.

Hoffman LM, Brooks SE, Schneck L
Neuroscience Center, Kingsbrook Jewish Medical Center, Brooklyn, NY, 11203
Biochim Biophys Acta; 665(3):359-361 1981

The composition of gangliosides in a cultured human fetal brain cell line was examined before and after SV-40 transformation. In contrast to most reports on other cell lines, an increase was observed in the complex ganglioside GM2 in the SV-40-transformed brain cell line. (Author abstract) (11 Refs)

13. ANALYSIS OF THE NONVIRAL ANTIGENS IMMUNOPRECIPITABLE BY SV40 T ANTIBODY FROM SV40-TRANSFORMED HUMAN/MOUSE HYBRID CELL LINES.

Stitt DT, Mangel WF
(c/o Mangel) Dept. Biochemistry, Univ. Illinois, Urbana, IL, 61801
Virology; 114(1):149-160 1981

The expression of nonviral antigens, ie, host-coded proteins with mol wt from 48,000-56,000 (48K-56K) immunoprecipitable from extracts of simian virus 40 (SV40)-infected or -transformed cells by anti-SV40 tumor antibody, was examined in four SV40-transformed human cell lines, one SV40-transformed mouse cell line, and eight SV40-transformed human/mouse somatic cell hybrid cell lines produced by fusion of SV40-transformed human cells with untransformed mouse cells. Differences were found in the patterns of expression of these viral induced proteins among the human cell lines, between the human and the mouse cell lines, and between the human/mouse somatic cell hybrid cell lines and the human lines from which they were derived. Two of the human cell lines expressed only a 55K nonviral antigen; two expressed both a 55K and a 52K nonviral antigen. The mouse cell line expressed both a 55K and a 48K nonviral antigen. Each hybrid cell line expressed a 55K and also a 48K nonviral antigen, except one. The BALB IV-8 hybrid cell line expressed 55K and 52K nonviral antigens. By comparing the tryptic peptides fractionated on cation exchange columns of the nonviral antigens we determined: (1) the 48K nonviral antigen in human/mouse hybrids is encoded by a mouse chromosome. (2) The 52K nonviral antigen in the BALB IV-8 human/mouse hybrid is encoded by a mouse chromosome. It is possibly a precursor to the mouse 48K protein because it contains one extra tryptic peptide in addition to those of the mouse 48K protein, because one peptide has an altered mobility and because the 52K protein is found, so far, only in a human/mouse hybrid that does not exhibit a 48K nonviral antigen. (3) The 55K nonviral antigen in the C57-VII-6 human/mouse hybrid is encoded by a human chromosome. In the BALB IV-8 human/mouse hybrid, there is a 55K protein encoded by a human chromosome and probably a 55K protein encoded by a mouse chromosome. (4) If the gene encoding the human 55K nonviral antigen is located on a single chromosome, it is located on human chromosome 7. (Author abstract) (25 Refs)

14. SV40 ANTITUMOR SERA THAT DIRECTLY IMMUNOPRECIPITATE SMALL-T-ASSOCIATED CELLULAR PROTEINS.

Rundell K, Yang YC
Dept. Microbiology-Immunology, Northwestern Univ. Medical Center, 303 E. Chicago Ave., Chicago, IL, 60611
Virology; 114(1):297-301 1981

Two cellular proteins, 56K and 32K, are immunoprecipitated in association with SV40 small-t antigen using antisera from animals bearing SV40-induced tumors. Antitumor sera have now been identified that immunoprecipitate these proteins directly from uninfected, as well as infected, cells. (Author abstract) (10 Refs)

15. HYBRIDIZATION OF A MOUSE T-CELL LYMPHOCYTE LINE (HB1) WITH A POLYOMA VIRUS-TRANSFORMED MOUSE FIBROBLAST LINE.

Berebbi M, Foa C, Barra Y, Simonetti J, Meyer G
Unite 119 de l'INSERM, F-13009 Marseille, France
Exp Cell Res; 134(2):445-456 1981

The establishment of permanent T-lymphocyte cell lines by transformation with DNA viruses has not yet been achieved. This paper reports the successful transfer of polyoma virus genome into T-lymphocyte cells by somatic hybridization. A T-lymphocyte clone, HB1, derived from (DBA/2J x AKR) spleen cells, isolated in vitro by cloning in semi-solid agar, was fused with a polyoma (Py) virus-transformed fibroblast (C3HPy, clone 1). The authenticity of the hybrid C3H/HB was established by chromosome and histocompatibility antigen studies. This initial population and the various clones retained T-lymphocyte characteristics such as morphological appearance, growth properties (suspension culture) and differentiation antigen (Thy 1-2). The hybrid cell line and the various clones presented all the characteristics of Py transformation. Namely, they carried the Py genome originating from the fibroblastic parent and maintained Py virus tumor-associated antigens (TSTA, TSSA and T antigens). In most respects, this hybrid population resembled the C3HPy/Cl1 parent and exhibited the same tumorigenicity. (Author abstract) (28 Refs)

16. EXPRESSION OF VIRAL DNA IN ADENOVIRUS TYPE 12-TRANSFORMED CELLS, IN TUMOR CELLS, AND IN REVERTANTS.

Schirm S, Doerfler W
Inst. Genetics, Univ. Cologne, Cologne, W. Germany
J Virol; 39(3):694-702 1981

The expression as cytoplasmic RNA of integrated human adenovirus Type 12 (Ad12) DNA in transformed and tumor cell lines and in revertants was investigated. The transformed and tumor cells contained multiple copies of the viral genome, 3 to 22 copies per cell in different cell lines. The integrated Ad12 DNA molecules persisted intact or nearly intact and in most cases colinear with the virion DNA. In the revertant cell lines, which were derived from cell line T637 (22 copies of Ad12 DNA per cell), all of the Ad12 DNA molecules were lost (line F10) or only one copy and a fraction of a second copy persisted (line TR12). The size classes map locations of

Ad12-specific cytoplasmic RNAs in three Ad12-transformed hamster cell lines (T637, HA12/7, and A2497-3), in two revertant lines (F10 and TR12), in one Ad12-induced hamster (CLAC3), and in one rat brain tumor line (RBT12/3) were determined. Cytoplasmic RNA from uninfected B3 hamster cells and from human KB cells productively infected with Ad12 served as controls. In the later contro l experiments, the RNA was isolated early or late postinfection. The expression of the integrated viral genomes in these lines was regulated by mechanisms more complicated than simple gene dosage effects. Using cloned fragments of Ad12 DNA as hybridization probes, we analyzed the cytoplasmic RNAs from the cell lines mentioned by electrophoresis on agarose gels, blotting, and DNA-RNA hybridization. For each transformed and tumor cell line, except for the revertants, several size classes of Ad12-specific cytoplasmic RNA were detected for the early E1, E2, and E4 regions of Ad12 DNA. Only cell lines A2497-3, T637, and RBT12/3 contained several size classes of cytoplasmic RNA homologous to the E3 region of Ad12 DNA. In preliminary experiments, we failed to detect the exp ression as cytoplasmic RNA of the so-called virus-associated RNA in transformed and tumor cell lines. Virus-associated RNA represents a population of low-mol-wt RNAs that map at around 30 fractional length units on the viral genome. (32 Refs)

17. INHIBITION OF ADENOVIRUS-TRANSFORMED CELL ONCOGENICITY BY ADENO-ASSOCIATED VIRUS.

Ostrove JM, Duckworth DH, Berns KI
Dept. Microbiology, Johns Hopkins Univ., Sch. Medicine, Baltimore, MD, 21205
Virology; 113(2):521-533 1981

Adeno-associated virus (AAV) has been shown to inhibit the oncogenicity and transforming ability of adenovirus (Ad). Using the H14b Ad-transformed hamster cell line, we have developed a model system in which to characterize the molecular events responsible for these inhibitory effects. AAV infection of H14b cells decreases their oncogenicity by approx threefold. The tumors that do arise are significantly smaller and have a longer latent period. AAV infection also alters the in vitro properties of the H14b cells, reducing cloning efficiency in methyl cellulose by 38-92% and saturation density by two- to four-fold. The molecular basis of this inhibition appears to be an inhibition of production of the 58K tumor antigen as determined by fluorescent antibody and immunoprecipitation studies. The reduction in the levels of tumor antigens are not due to alterations or loss of the Ad genome within H14b cells but may reflect an alteration in transcription or processing of Ad-specific mRNA. AAV infection of H14b cells does not lead to detectable levels of AAV DNA synthesis, but some AAV transcripts are made. This is the first instance in which AAV transcription has been detected in an Ad-transformed cell line. (Author abstract) (42 Refs)

18. SITE OF LINKAGE BETWEEN ADENOVIRUS TYPE 12 AND CELL DNAS IN HAMSTER TUMOUR LINE CLAC3.

Deuring R, Winterhoff U, Tamanoi F, Stabel S, Doerfler W
Inst. Genetics, Univ. Cologne, Cologne, W. Germany
Nature; 293(5827):81-84 1981

The total DNA of cell line CLAC3, an adenovirus type 12 (Ad12)-induced hamster t umor line carrying 5 copies of the Ad12 genome, was cleaved with restriction endonuclease EcoRI; fragments larger than 4.0 kilobases (fragment D) were selected by velocity sedimentation; fragment D was ligated to the arms of bacteriophage lambda-dcrivative vector; phage containing DNA sequences derived from the termini of AD12 DNA were selected by using the terminal EcoRI fragments A and C as probes; and recombinant phage were purified and propagated to yield large amounts of recombinant DNA for characterization. Three plaques of recombinant lambda phage containing DNA sequences homologous to the left terminus of Ad12 were isolated, and the DNA from them characterized by numerous techniques, including restriction endonuclease cleavage, Southern blotting, and DNA-DNA hybridization. The nucleotide sequence at the junction between the left terminus of the Ad12 DNA and the hamster cellular DNA is preserved beginning with base pair 46 of the Ad12 DNA, which occupies position 83 of the recloned fragment; the first 82 base pairs of the fragment are not viral but contain scattered 8-to 11-base-pair homologies to distant sequences within the first 2,722 left terminal base pairs of Ad12 DNA. Computer analysis revealed regions of dyad symmetry with only a few mismatched bases between base pairs 35 and 54 and 71 and 90 that straddle the site of the junction and permit the construction of a stemmed loop with 15 matched and 4 unmatched pase pairs in the stem and 16 nucleotides in the loop. The stability of this loop and its possible biological function(s) are still unknown. (23 Refs)

19. BIOCHEMICAL STUDIES ON BOVINE ADENOVIRUS TYPE 3. V. SOME PROPERTIES OF MOUSE CELLS TRANSFORMED WITH VIRAL DNA FRAGMENTS.

Niiyama Y, Sasada R, Igarashi K, Kurokawa T, Sugino Y
Biological Res. Lab., Central Res. Div., Takeda Chemical Industries, Ltd., Osaka 532, Japan
Cell Struct Funct; 6(2):121-131 1981

Tumorigenicity and cell-surface properties were studied in mouse A31 cells (a BALB/3T3 clone) transformed by fragments of the bovine adenovirus type 3 (BAV3): the EcoRI D fragment (3.6 to 19.7 map units; TrD1 cells) or the HindIII J-E ligated fragment (0 to 11.9 map units; TrJE6 cells). DNA-DNA reassociation kinetics experiments showed that both TrD1 and TrJE6 cells contained copies of the transforming region of BAV3. Hybridization experiments between RNA from transformed cells and BAV3 DNA showed that BAV3-specific RNA was synthesized in the transformed cells. Biochemically, both transformed cell lines showed increased uptake of 2-deoxyglucose compared with that by normal A31 cells, agglutination by plant lectins (concanavalin-A, wheat germ agglutinin, castor bean agglutinin, and Wistaria floribunda agglutinin) with different sugar specificities at lower concentrations of lectins than needed to agglutinate A31 cells, and higher activity of plasminogen activator than present in A31 cells. Both transformed cell lines produced tumors within 2 mo when inoculated into mice (10(3)-10(7) cells/mouse), whereas 10(7) A31 cells did not produce tumors. These results indicate that the altered properties of the transformed cells examined in these studies are directly or indirectly controled by the gene(s) located between map units 3.6 and 11.9 of the BAV3 genome. (32 Refs)

20. UNINTEGRATED VIRAL DNA SEQUENCES IN A HAMSTER TUMOR INDUCED BY BOVINE PAPILLOMA VIRUS.

Moar MH, Campo MS, Laird HM, Jarrett WF
Dept. Zoology, Univ. Edinburgh, Edinburgh EH9 3JT, Scotland
J Virol; 39(3):945-949 1981

A fibrosarcoma was induced in a hamster by bovine papilloma virus Type 2 (BPV2). The content of BPV2 DNA sequences was measured by DNA-DNA and cRNA-DNA hybridizations. The tumor contained approx 300 BPV2 genome

equivalents per cell. Southern blot hybridization indicated that the viral DNA was in free form, the entire genome most likely being present. In situ hybridization with BPV2 cRNA showed that multiple genome copies were present in each cell. Neither virus particles nor virus coat antigens could be detected in the tumor. A cell line was established from the fibrosarcoma, and the cells contained multiple copies of the BPV2 genome. The latter was in free form, and all of the DNA sequences appeared to be present in multiple copies and in all cells. An extensive search failed to reveal the presence of virus or viral antigens. (Author abstract) (29 Refs)

21. BIOCHEMICAL TRANSFORMATION OF LM(TK-) CELLS BY HYBRID PLASMIDS CONTAINING THE CODING REGION OF THE HERPES SIMPLEX VIRUS TYPE 1 THYMIDINE KINASE GENE.

Kit S, Qavi H, Hazen M, Trkula D, Otsuka H
Div. Biochemical Virology, Baylor Coll. Medicine, Houston, TX, 77030
Virology; 113(2):452-464 1981

Recombinant plasmid pAGO codes for herpes simplex virus Type 1 (HSV-1) thymidine kinase (TK) and consists of a 2-kbp HSV-1 DNA fragment inserted at the unique PvuII cleavage site of plasmid pBR322. A hybrid plasmid, designated pMH110, has been derived from plasmid pAGO by deleting the 1689-bp pBR322 nucleotide sequence of pAGO, which extends from the BamHI to the PvuII cleavage site, and the 250-bp HSV-1 nucleotide sequence of pAGO, which extends from the PvuII to the BglII cleavage site. Plasmid pMH110 biochemically transformed LM(TK-)cells to the TK+ phenotype. The biochemically transformed cell lines had the following properties: (i) they were resistant to the growth-inhibiting effects of 1 mM thymidine; and (ii) they expressed an HSV-1-specific TK activity. This HSV-1 TK activity was purified after labeling biochemically transformed cell lines [LM(TK-)/TF pMH110 E2 and LM(TK-)/TF pMH110 Hc2] with [35S]methionine. Immunoprecipitation experiments revealed that the TK polypeptides made in the biochemically transformed cells ha d mol wt of about 39,000 to 40,000, which are about the same as the mol wt of the TK polypeptides previously purified from HSV-1-infected LM(TK-) cells and other biochemically transformed cell lines. The experiments support the hypothesis that the functional coding region of the HSV-1 TK gene is 3' to the BglII cleavage site, and they also suggest that the HSV-1 TK messenger RNA may have been initiated in cells transformed by HincII- and EcorRI-cleaved pMH110 DNA at a site in cellular (or plasmid) DNA upstream from the HSV-1 DNA BglII cleavage site. (Author abstract) (29 Refs)

22. INDUCTION OF UTERINE CANCER WITH INACTIVATED HERPES SIMPLEX VIRUS, TYPES 1 AND 2.

Wentz WB, Reagan JW, Heggie AD, Fu YS, Anthony DD
Reproductive Biology, Case Western Reserve Univ. Sch. Medicine, Cleveland, OH, 44106
Cancer; 48(8):1783-1790 1981

A series of studies were performed to evaluate the oncogenic potential of inactivated herpes simplex viruses Types 1 (HSV-1) and 2 (HSV-2) in the mouse cervix. HSV-1 or HSV-2 prepared in HEp-2 cells cultured and inactivated by exposure to formalin or UV light was applied to the mouse cervix for periods ranging from 20 to 90 wk. Control mice were exposed for the same period to control fluids. Vaginal cytologic preparations from all animals were examined weekly to detect epithelial abnormalities. Animals were sacrificed and histopathological studies were carried out when cellular changes seen on vaginal smears resembled those indicative of premalignant or malignant changes as previously established in a similar model system using coal tar hydrocarbons. Other animals were exposed for periods up to 90 wk, or until there was cellular evidence of invasive cancer. Cytologic and histologic materials were coded and evaluated without knowledge of whether they were from virus-exposed or control animals. Premalignant and malignant cervical lesions similar to those that occur in women were encountered in 78 to 90% of the virus-exposed animals. All controls were normal. Invasive cancer were detected in 24 to 60% of the animals and dysplasia was found in 18 to 66%. The yield of invasive cancer was twice as great after exposure to UV-inactivated HSV-2 as compared with formalin-inactivated virus. Various histologic grades of carcinoma of the cervix and endometrium were found. No primary lesions were found in the vagina or ovaries. (Author abstract) (10 Refs)

23. CONSTRUCTION AND ISOLATION OF A TRANSMISSIBLE RETROVIRUS CONTAINING THE SRC GENE OF HARVEY MURINE SARCOMA VIRUS AND THE THYMIDINE KINASE GENE OF HERPES SIMPLEX VIRUS TYPE 1.

Wei CM, Gibson M, Spear PG, Scolnick EM
Lab. Tumor Virus Genetics, NCI, Bethesda, MD, 20205
J Virol; 39(3):935-944 1981

We constructed lambda recombinants containing the Harvey murine sarcoma virus genome and the thymidine kinase (tk) gene of herpes simplex virus Type 1 linked to each other. The tk gene was located in a position downstream from both the long terminal repeat and the src gene of Harvey murine sarcoma virus. The DNAs of the lambda recombinants were used to transfect NIH3T3 mouse fibroblasts in order to obtain Harvey murine sarcoma virus DNA-induced foci of transformed cells. The transformed foci were superinfected with a helper-independent retrovirus, and new individual retroviruses were isolated from the superinfected foci. The new viruses could induce focus formation on NIH3T3 cells and could convert NIH3T3(TK-) cells into TK+ cells by carrying the herpes simplex virus Type 1 tk gene into the TK- cells. From virus-infected cells, we isolated nonproducer foci on NIH3T3 cells and TK+ transformants on NIH3T3(TK-) cells containing one such new viral genome coding for the dual properties. The new retroviral sequence in the nonproducer cells could be rescued into virus particles at high titers by superinfection with a helper-independent retrovirus. A hybridization analysis indicated that the recombinant virus contained both the Harvey murine sarcoma virus src sequence and the tk gene sequence in a single RNA species approx 4.9 kilobases long. We concluded that retroviruses can be used as true vectors for genes other than genes that lead to oncogenesis. (Author abstract) (50 Refs)

24. COCARCINOGENIC EFFECT OF VACCINIA VIRUS AND HERPES SIMPLEX VIRUS IN TISSUE CULTURE.

Mazurenko NP, Merekalova ZI, Pavlish OA, Bykovskii AF, Kurtsman MIa, Mazurenko NN
All-Union Cancer Res. Center, Moscow, USSR
Vestn Akad Med Nauk SSSR; (7):3-10 1981

Cocarcinogenic effect of vaccinia virus (VV) and herpes simplex virus (HSV) was studied in transplantable murine cell lines. The cultured cells of embryonal tissue of BALB/c mice were infected with VV, and the expression of structural protein p30 was assayed by indirect immunofluorescence reaction. Specific cytoplasmatic fluorescence was recorded in 15-20% of

cells. Max fluorescence was observed 48-72 hr after infection, which corresponded to the onset of Rauscher leukemia virus replication. HSV-2 caused malignant transformation of BALB/3T3 cells. Inoculation of irradiated BALC/c mice with transformed cells (subclones 333-0 and 333-1) produced tumors in 25% and 80% of the animals. Transformed cells expressed the endogenous murine retrovirus Type 3. (29 Refs) (In RUS)

VIRUS REPLICATION AND EXPRESSION

25. A PARANUCLEAR EXTRACT CONTAINS A UNIQUE SET OF VIRAL TRANSCRIPTS LATE IN SV40 INFECTION.

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Virology; 113(2):663-671 1981

Late in infection with SV40, CV1 cells are fractionated into cytoplasmic, paranuclear, and nuclear fractions. Viral RNA is extracted from these fractions and examined by the nuclease S1 assa y. The paranuclear fraction, which is partially enriched for pore complexes and inner nuclear membranes, was observed to contain a specific, unique, and previously undescribed set of three late transcripts. The nuclease S1-generated 5' ends (bodies) of these transcripts are at 0.820, 0.837, and 0.852 mu. These transcripts appear to be spliced. Possible explanations for the localization and function of these molecules are discussed. (Author abstract) (20 Refs)

26. INHIBITION OF SV40 REPLICATION IN SIMIAN CELLS BY SPECIFIC PBR322 DNA SEQUENCES.

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Dept. Molecular Biology, Univ. California, Berkeley, CA, 94720
Nature; 293(5827):79-81 1981

Dual-host replicons such as a unit consisting of a bacterial plasmid and genes from a mammalian virus could be of considerable utility in cloning specific genes that are properly the part of the mammalian host's genome and in amplifying the expression of specific cloned eukaryotic genes. Such a dual-host replicon exists: SV40-pBR322, which consists of an Escherichia coli plasmid and the SV40 genome, but it replicates poorly after transfection of simian cells and also shows a reduced ability to retransform E coli following isolation after transfection. Initial experiments showed that this recombinant plasmid did not replicate to any significant extent in transfected CV-1 simian cells and replicated far less well than wild type SV40 in COS7 cells (a permissive strain of CV-1). Two types of experiment showed that these results were not due to a lack of normal A gene expression in the recombinant plasmid but rather were likely due to a cis-acting element present in the chimeric molecule: (1) intact SV40 DNA enzymatically excised from the recombinant could replicate like wild type SV40 in both CV-1 and COS7 cells and (2) when a partial head-to-tail tandem duplication of SV40 was transfected into either CV-1 or COS7 cells, excision of genomic length viral DNA occurred leading to accumulation of detectable form I and form II SV40 DNA. Because SV40 replication in simian cells produces defective viral DNA, plasmid progeny extracted from COS7 cells were used to transform E coli cells; the plasmid DNA was purified from the bacterial hosts and examined for molecules with either a selective transforming ability or a higher replication rate in simian cells: restriction analysis revealed 10 such plasmids that had undergone overlapping deletions near the pBR322 origin of replication, 5 of which replicated in both COS7 and CV-1 cells at rates equal to that of authentic SV40 DNA. This result shows that certain pBR322 sequences interfered with SV40 replication in simian cells. The structures of some of these deletions and their genetic consequences are discussed. The molecular basis for the described restrictions is not known. (18 Refs)

27. DOES SIMIAN VIRUS 40 T ANTIGEN UNWIND DNA?

Myers RM, Kligman M, Tjian R
Dept. Biochemistry, Univ. California, Berkeley, CA, 94720
J Biol Chem; **256(19):10156-10160 1981**

Recently developed techniques allow rapid and accurate determination of protein-induced DNA unwinding. We have used such techniques to determine whether SV40 T antigen unwinds DNA. Assays were performed with purified wild type T antigen, D2 protein, and the SV80 T protein over a wide range of protein concentrations. Additionally, several DNA templates were used, including SV40 DNA, pBR322 DNA, and two plasmid DNAs containing one and three copies of the SV40 origin of replication. None of the purified proteins showed unwinding activity either in the presence or absence of ATP and Mg++. As a positive control, we found that unwinding of pBR322 DNA by Escherichia coli RNA polymerase occurred under identical assay conditions. Protein-DNA interactions that involve unwinding of the helix are sensitive to the superhelical density of the DNA. Using a filter binding assay, we found that the efficiency of the binding of T antigen to the viral origin of replication does not depend upon the topological state of the DNA. This result further supports our conclusion that SV40 T antigen is not an unwinding protein. (Author abstract) (32 Refs)

28. DNA REPLICATION-MEDIATED RECOMBINATION OF MOLECULES OF ADENOVIRUS 2 DNA.

Wolgemuth DJ, Hsu M-T
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Proc Natl Acad Sci USA; 78(8):5076-5080 1981

Molecules of adenovirus 2 DNA were isolated as nucleoprotein complexes late in infection of HeLa cells and examined by electron microscopy. Some were interpreted as representing intermediates in recombination events. [illegible] were consistent with predicted intermediates according to the model for genetic recombination proposed by Meselson and Radding and constitute physical evidence from animal cells in support of this model. (Author abstract) (16 Refs)

29. NONSTRUCTURAL PROTEINS OF HERPES SIMPLEX VIRUS. II. MAJOR VIRUS-SPECIFIC DNA-BINDING PROTEIN.

Powell KL, Littler E, Purifoy DJ
Dept. Microbiology, Univ. Leeds, Leeds LS2 9JT, England
J Virol; 39(3):894-902 1981

The major herpes simplex virus Type 2 DNA-binding infected cell-specific polypeptides 11 and 12 have been purified to homogeneity from extracts of virus-infected cells. Monospecific antiserum to the purified protein has been made and used to examine virus temperature-sensitive mutants for defects in the synthesis of the protein and to probe virus DNA synthesis in isolated chromatin. The purified protein acted directly on a polydeoxyadenylic acid-polydeoxythymidylic acid helix, reducing

its melting temperature. The results indicated that the protein functions in virus DNA synthesis. (Author abstract) (24 Refs)

VIRAL GENETICS

30. PHYSICAL MAPPING OF DRUG RESISTANCE MUTATIONS DEFINES AN ACTIVE CENTER OF THE HERPES SIMPLEX VIRUS DNA POLYMERASE ENZYME.

Knopf KW, Kaufman ER, Crumpacker C
Medical Res. Council Virology Unit, Univ. Glasgow, Glasgow, Scotland
J Virol; 39(3):746-757 1981

The genome structures of herpes simplex virus Type 1 (HSV-1)/HSV-2 intertypic recombinants have been previously determined by restriction endonuclease analysis, and these recombinants and their parental strains have been employed to demonstrate that mutations within the HSV DNA polymerase locus induce an altered HSV DNA polymerase activity, exhibiting resistant to three inhibitors of DNA polymerase. The viral DNA polymerases induced by two recombinants and their parental strains were purified and shown to possess similar mol wt (142,000 to 144,000) and similar sensitivity to compounds which distinguish viral and cellular DNA polymerases. The HSV DNA polymerases induced by the resistant recombinant and the resistant parental strain were resistant to inhibition by phosphonoacetic acid, acycloguanosine triphosphate, and the 2',3'-dideoxynucleoside triphosphates. The resistant recombinant (R6-34) induced as much acycloguanosine triphosphate as did the sensitive recombinant (R6-26), but viral DNA synthesis in infected cells and the viral DNA polymerase activity were not inhibited. The 2',3'-dideoxynucleoside-triphosphates were effective competitive inhibitors for the HSV DNA polymerase, and the Ki values for the four 2',3'-dideoxynucleoside triphosphates were determined for the four viral DNA polymerases. The polymerases of the resistant recombinant and the resistant parent possessed a much higher Ki for the 2',3'-dideoxynucleoside triphosphates and for phosphonoacetic acid than did the sensitive strains. A 1.3-kilobase-pair region of HSV-1 DNA within the HSV DNA polymerase locus contained mutations which conferred resistance to three DNA polymerase inhibitors. This region of DNA sequences encoded for an amino acid sequence of 42,000 mol wt and defined an active center of the HSV DNA polymerase enzyme. (Author abstract) (29 Refs)

IMMUNOLOGICAL STUDIES

31. ANTIBODIES SPECIFIC FOR THE POLYOMA VIRUS MIDDLE-SIZE TUMOR ANTIGEN.

Walter G, Hutchinson MA, Hunter T, Eckhart W
Institute fur Immunobiologie der Albert-Ludwigs-Universitat, 7800 Freiburg i. Br., W. Germany
Proc Natl Acad Sci USA; 78(8):4882-4886 1981

We have obtained antibodies specific for the polyoma virus middle-size tumor antigen (middle T antigen) by immunizing rabbits with a synthetic peptide, Lys-Arg-Ser-Arg-His-Phe, corresponding to the six carboxy-terminal amino acids of the middle T antigen predicted from the nucleotide sequence of polyoma DNA. The antipeptide serum precipitates the polyoma middle T antigen but not the small or large tumor antigens, and precipitation is inhibited in the presence of the peptide. Two cellular proteins, 30,000 and 26,000 daltons, are also precipitated specifically by the antipeptide serum and may have amino acid sequences related to the peptide. Two other cellular proteins, 33,000 and 25,000 daltons, are precipitated only in the presence of the peptide and may associate with it in cell extracts. Antisera directed against synthetic peptides are likely to be important in various ways, including the production of antibodies directed against particular determinants and the recognition of unknown proteins whose genes have been analyzed. (Author abstract) (21 Refs)

32. EPSTEIN-BARR VIRUS STRAIN- AND GROUP-SPECIFIC ANTIGENIC DETERMINANTS DETECTED BY MONOCLONAL ANTIBODIES.

Mueller-Lantzsch N, Georg-Fries B, Herbst H, Zur Hausen H, Braun DG
Institut fur Virologie, Zentrum fur Hygiene der Universitat Freiburg, Hermann-Herder-Strasse 11, 7800 Freiburg, W. Germany
Int J Cancer; 28(3):321-327 1981

In order to differentiate Epstein-Barr virus (EBV) strains by serological markers we prepared hybridomas producing monoclonal antibodies against polypeptides of the QIMR-WIL EBV strain. These monoclonal antibodies were screened by indirect immunofluorescence techniques. Immunoprecipitation, using EBV-positive monoclonal antibodies and the protein A method, of 125I-labeled polypeptides from purified QIMR-WIL EBV particles, revealed that a series of monoclonal antibodies against the four main surface and envelope polypeptides precipitating p340, p340/p240, p140, and p80 were obtained. When 125I-labeled polypeptides from purified P3HR-1 and B95-8 EBV particles were immunoprecipitated, it could be demonstrated that several anti-p340 (QIMR-WIL) antibodies recognized strain-specific antigenic determinants, while anti-p340/p240 (QIMR-WIL) as well as anti-p140 (QIMR-WIL) antibody clones reacted with antigenic sites which are in common wither among B95-8 or in addition to P3HR-1 polypeptides, Thus, monoclonal antibodies now provide a serological basis for EBV typing. (Author abstract) (31 Refs)

33. INCREASED SUSCEPTIBILITY TO CYTOMEGALOVIRUS INFECTION IN BEIGE MUTANT MICE.

Shellam GR, Allan JE, Papadimitriou JM, Bancroft GJ
Dept. Microbiology, Univ. Western Australia, Nedlands 6009, Australia
Proc Natl Acad Sci USA; 78(8):5104-5108 1981

Mice homozygous for the beige gene (bg/bg) are a homologue of the Chediak-Higashi syndrome of man and are known to be selectively defective in natural killer (NK) cells. We have compared the susceptibility of bg/bg and bg/+ C57BL/6J mice to infection with murine cytomegalovirus (MCMV). Beige mice are more susceptible to lethal infection and develop 33-to 43-fold higher virus titers in the liver, spleen, and kidney than do bg/+ mice after a sublethal infection, although virus replication is the same in vitr o in cultured fibroblasts or epithelial cells from these mice. Inoculation with a sublethal dose of virus stimulates a NK cell response, although this is lower in bg/bg mice despite higher titers of interferon type 1 than in bg/+. A dose of MCMV that is lethal only to bg/bg augments cytotoxicity within 12 hr in bg/+ mice, whereas cytotoxicity in bg/bg remains very low. In bone marrow chimeras, recipients of bg/bg marrow were more susceptible to MCMV and

had lower NK cell responses after virus inoculation than did recipients of marrow from bg/+ donors. The greater susceptibility of beige mice to the virus suggests that NK cells may contribute to resistance early in MCMV infection. (Author abstract) (26 Refs)

34. DIFFERENTIAL IMMUNOLOGIC REACTIVITY AND PROCESSING OF GLYCOPROTEINS GA AND GB OF HERPES SIMPLEX VIRUS TYPES 1 AND 2 MADE IN VERO AND HEP-2 CELLS.

Pereira L, Dondero D, Norrild B, Roizman B
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Proc Natl Acad Sci USA; 78(8):5202-5206 1981

Herpes simplex virus Types 1 and 2 (HSV-1 and HSV-2, respectiely) specify five major glycoproteins designated as gA, gB, gC, gD, and gE. Previous studies have shown that gA and gB differ in electrophoretic mobility but not in reactivity with antisera prepared to each of these glycoproteins. Moreover, gA and gB of HSV-1 crossreact in serologic tests with the corresponding glycoproteins of HSV-2. In this paper, we report on the reactivities of gA and gB of HSV-1 and HSV-2 with 24 independently derived monoclonal antibodies reactive with these antigens. Our results show the following: (i) Electrophoretic mobilities of HSV-1 and HSV-2 glycoproteins gA and gB made in HEp-2 cells are significantly less than those made in Vero cells. (ii) All monoclonal antibodies precipitated both gA and gB made in infected HEp-2 cells. These include 12 monoclonal antibodies that neutralized virus and 12 that did not. (iii) HSV-2 glycoproteins gA and gB made in HEp-2 cells contain type-specific domains. However, monoclonal antibodies produced by one clone directed to these domains did not react with glycoproteins made in Vero cells. (iv) Lysates of infected Vero cells contain three lower mol wt polypeptides that also reacted with monoclonal antibodies directed to glycoproteins gA and gB. These polypeptides are virus specific inasmuch as those specified by HSV-1 differ in electrophoretic mobilities from those specified by HSV-2. These polypeptides are absent in lysates of HEp-2 cells. (Author abstract) (21 Refs)

OTHER RELATED STUDIES

35. STAPHYLOCOCCAL PROTEIN A ENHANCES NATURAL KILLING ACTIVITY AGAINST LYMPHOID TUMOR CELL LINES.

Patel PC, Stefanescu-Soare I, Menezes J
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Int J Cancer; 28(3):277-284 1981

Protein A (pA), was found to augment the natural killing (NK) activity of peripheral blood lymphocytes against Burkitt's lymphoma-derived Raji and Daudi cells. This pA-enhanced NK activity was determined using the 51 Chromium release microcytotoxicity assay. It was found that the increased NK activity was dependent on the concentration of pA used. In addition, the pA-enhanced NK activity was dependent on the time of incubation of pA with the effector and target cell mixture. Pretreatment of effectors for 16 hr or more dramatically increased the NK activity against a wide range of tumor targets of different origin and possessing either B, T or null cell surface characteristics. The presence of the Epstein-Barr virus (EBV)-genome in the target cells did not influence the pA-enhanced NK activity. Nylon-wool column-fractionated, non-adherent lymphocytes (enriched in NK cells) showed an increased NK activity in the presence of pA than unfractionated lymphocytes. Pretreatment of certain targets with pA rendered them more sensitive to NK lysis. Interferon production was readily detected in supernatants from cultures of pA-treated effectors or effector-target mixtures, but not in supernatants from pA-treated target cultures tested. Results of different physicochemical treatments indicate that the pA-induced interferon is of gamma type (Type II). Thus, the data presented suggest that the pA-induced enhancement of NK activity is most likely mediated by the pA-induced interferon. (Author abstract) (26 Refs)

36. HODGKIN'S DISEASE: ESTABLISHMENT AND CHARACTERIZATION OF FOUR IN VITRO CELL LINES.

Diehl V, Kirchner HH, Schaadt M, Fonatsch C, Stein H, Gerdes J, Boie C
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J Cancer Res Clin Oncol; 101(1):111-124 1981

Four in vitro cells established from the pleural effusions of two Hodgkin's disease (HD) patients and from the blood and bone marrow of a third were characterized morphologically, immunologically, and biochemically. The malignant nature of the cultured cells was confirmed by the presence of structural and numerical chromosome abnormalities, the monoclonality of marker chromosomes, and the lack of Epstein-Barr virus-specific antigens. The cells gave rise to tumors in nude mice following intracranial transplantation and induced sc tumors when embedded on plasma dots before transplantation. All four lines were positive for Ia-like antigens, T-cell receptors, acid phosphatase, and acid esterase; they were negative for lysozyme peroxidase, chloracetate esterase, surface Ig or cytoplasmic Ig (CIg), and receptors for complement 3b, complement 3d, IgG-FC, and mouse or sheep RBC. Except for the lack of CIg in the in vitro cells, the marker pattern corresponded to that of Hodgkin's and Sternberg-Reed cells in biopsy mat erial. The phenotype was not that of B cells, T cells, myeloid cells, monocytes, or macrophages. (26 Refs)

37. ABILITY OF VACCINIA VIRUS TO INDUCE EXPRESSION OF RETRAVIRUS P30 IN CONTINUOUS MICE CELL LINES.

Merekalova ZI, Mazurenko NP, Rapp F, Turner N
Lab. Virus Carcinogenesis, Cancer Res. Center, Moscow, USSR
Biull Eksp Biol Med; 91(6):710-712 1981

Ability of vaccinia virus to induce expression of the major structural protein p30 of Rauscher leukemia virus (RLV) and Moloney sarcoma virus was studied in mice continuous cell lines (NclAcl 10 and 3T3 BALB). Immunoperoxidase technique showed marked fluorescence in cells infected with RLV and vaccinia virus. (13 Refs) (In RUS)

38. INDUCTION OF URACIL-DNA GLYCOSYLASE AND DUTP NUCLEOTIDOHYDROLASE ACTIVITY IN HERPES SIMPLEX VIRUS-INFECTED HUMAN CELLS.

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J Biol Chem; 256(19):9834-9837 1981

HeLa BU cells infected with either the Type 1 or the Type 2 forms of herpes simplex virus show an increase in the activities of uracil-DNA glycosylase and dUTP nucleotidohydrolase. Under optimal conditions, uracil-DNA glycosylase activity increases approx 40-fold in HSV Type 2-infected cells. In herpes simplex virus (HSV) Type 1-infected cells, uracil-DNA glycosylase activity increases only 6-fold. At a KCl concentration of 100 mM, uracil-DNA glycosylase derived from HSV Type 2-infected cells is activated two-fold, while the glycosylase extracted from mock infected HeLa BU cells is inhibited almost 90% at 100 mM KCl. dUTP nucleotidohydrolase activity increases four-fold and three-fold, respectively, in HSV Type 1- and and HSV Type 2-infected HeLa BU cells. Nondenaturing polyacrylamide gel electrophoresis of extracts derived from the Type 1-and Type 2-infected cells indicates distinct electrophoretic mobilities from the host cell enzyme. dUTP nucleotidohydrolase RF values for the mock infected cells, HSV Type 1, and HSV Type 2 are 0.5, 0.25, and 0.33, respectively. Serum from rabbits immunized against cells infected with herpes simplex virus Type 1 or Type 2 specifically neutralizes the dUTPase and uracil-DNA glycosylase activities extracted from herpes simplex virus-infected cells. This serum does not neutralize dUTPase or uracil-DNA glycosylase activity derived from mock infected cells. (Author abstract) (26 Refs)

39. AUGMENTATION IN THE INFECTIVITY OF SV40 VIRUS DNA BY AMPHOTERICIN B METHYL ESTER. BRIEF REPORT.

Borden EC, McBain JA, Martin J, Walker D
Dept. Human Oncology, Univ. Wisconsin Clinical Sciences Center, Madison, WI, 53792
Arch Virol; 69(2):161-165 1981

The methyl ester of amphotericin B (100 ug/ml) enhanced the infectivity of SV40 virus DNA for CV-1 cells up to 300-fold. Amphotericin B methyl ester may prove useful for augmenting cellular penetration of macromolecules and for probing biological mechanisms. (Author abstract) (15 Refs)

40. CHANGES IN ACTIVITY OF OXIDATIVE ENZYMES IN THYMUS CELLS OF CBA MICE DURING CARCINOGENESIS INDUCED BY SIMIAN ADENOVIRUS SA7(C8).

Ageenko AI, Avtandilov GG, Kruglova IS, Sviridova IK
Lab. Virology, P. A. Hertsen Res. Oncological Inst., Moscow, USSR
Biull Eksp Biol Med; 91(7):80-82 1981

The newborn CBA mice were inoculated sc with simian adenovirus SA7(C8). On day 60 after inoculation, 95% of animals developed hemangiopericytoma. Histochemical examination of thymus specimens showed progressive decrease of activity of dehydrogenases. The decrease was first detected on day 30 of the latent period and was the most pronounced in tumor-bearing mice. (13 Refs) (In RUS)

41. BIOLOGICAL ACTIVITIES AND MODES OF ACTION OF 9-ALPHA-D-ARABINOFURANOSYLADENINE AND 9-ALPHA-D-ARABINOFURANOSYL-8-AZAADENINE.

Bennett LL, Allan PW, Shaddix SC, Shannon WM, Arnett G, Westbrook L, Drach JC, Relake CM
Biochemistry Res. Dept., Southern Res. Inst., 2000 Ninth Ave. South, Birmingham, AL, 35255
Biochem Pharmacol; 30(16):2325-2332 1981

From earlier studies it is known that 9-alpha-D-arabinofuranosyladenine (alpha-araA) and 9-alpha-D-arabinofuranosyl-8-azaadenine (alpha-ara-8-azaA) have in vitro antiviral activity, are cytotoxic, and are metabolized in mammalian cells to the triphosphates. This study was designed to compare the in vivo antiviral activities of these compounds and their loci of action with those of 9-beta-D-arabinofuranosyladenine (beta-araA). The latter compound selectively inhibits DNA synthesis in intact cells, and its triphosphate is a known inhibitor of DNA polymerases and ribonucleotide reductase. Whereas beta-araA was significantly effective in the treatment of systemic herpes simplex virus Type 1 (HSV-1) infections in mice, alpha-araA and alpha-ara-8-azaA were therapeutically ineffective. Alpha-AraATP at a concentration of approx 1 mM did not inhibit (1) DNA polymerases present in crude extracts of cultured HEp-2 cells; (2) DNA polymerases present in extracts of KB cells; (3) partially purified DNA polymerase-alpha from mouse embryo cells; or (4) DNA polymerases induced by HSV-1 and HSV-2. DNA polymerase-beta from mouse embryo cells was inhibited to a small extent by 10(-4) M alpha-araATP. In contrast, all of these enzymes were inhibited by beta-araATP at a concentration of 10(-5) M (as shown in these or in earlier studies). The reductions of CDP and UDP by ribonucleotide reductase from L1210 cells were not inhibited by alpha-araATP (approx 10(-3) M), whereas beta-araATP produced 70-80% inhibition at this concentration. In cultured HEp-2 cells, alpha-ara-8-azaA inhibited the incorporation of thymidine, uridine, and f ormate into macromolecules, but it was without effect on the incorporation of adenine and hypoxanthine, and produced marginal inhibition of the incorporation of leucine. alpha-Ara-8-azaA produced a dose-dependent inhibition of the accumulation of [14C]formyl-glycinamide ribonucleotide in HEp-2 cells treated with azaserine and [14C]formate. These results indicate that the alpha-nucleosides inhibit nucleic acid synthesis by mechanisms different from those of beta-ara-A. (Author abstract) (32 Refs)

42. PRODUCTION OF INTERFERON BY AN SV40-TRANSFORMED MACROPHAGE LINE, BB-W-531-2.

Takayama H, Tanigawa T, Takagi A
Dept. Bacteriology, Tottori Univ., Sch. Medicine, Yonago, Tottori 683, Japan
Microbiol Immunol; 25(7):683-692 1981

Interferon (IF) production during the phenotypic transition between the nonmacrophage and macrophage states of Simian virus-40 (SV40)-transformed cells was examined. The ability of SV40-transformed cells to produce IF following treatment with various viral and nonviral inducers was studied. Stationary SV40-transformed macrophages (BB-W-531-2 BALB/cAnN mouse cells) culture-split and cultivated on tissue culture dishes (SP method) produced IF without exogenous stimulation, although only in the growing phase and not in the stationary phase. Stationary transformed cells grown on bacteriologic dishes (BP method) or growing cells grown on tissue culture dishes (SGP method) did not produce a significant level of IF throughout the culture period. When the cells that were subcultured by the BP method were treated with various inducers

(penicillin-attenuated Streptococcus pyogenes, lipopolysaccharide, and polyinosinate-polycytidylate), large quantities of IF were produced. Cells subcultured according the SGP method produced no significant IF after identical exposure to inducers. The IF induction in cells subcultured according to the BP method following lipopolysaccharide administration was dose dependent. The characteristics of the BB-W-531-2 cell-produced IF-like substance are described. The results suggested that the ability of BB-W-531-2 cells to produce IF is associated with the expression of several macrophage properties. (20 Refs)

43. SIMILARITIES BETWEEN FIBROBLAST-DERIVED GROWTH FACTOR AND PLATELET-DERIVED GROWTH FACTOR.

Dicker P, Pohjanpelto P, Pettican P, Rozengurt E
Imperial Cancer Res. Fund, Lincoln's Inn Fields, London WC2A 3PX, England
Exp Cell Res; 135(1):221-227 1981

Platelet-derived growth factor (PDGF), one of the most potent mitogens in serum for non-transformed cells, shares many biological and physical properties with fibroblast-derived growth factor (FDGF), a polypeptide produced by BHK cells transformed by SV40. Thus FDGF and PDGF have biological activity which is recoverable from sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, at positio ns indicating similar mol wt. Further, the biological activity of both factors is heat-stable but sensitive to mercaptoethanol. FDGF and PDGF have similar abilities to induce DNA synthesis synergistically in the presence of either insulin, epidermal growth factor (EGF), vasopressir or colchicine. In contrast to other growth factors, (i) either FDGF or PDGF can induce DNA synthesis in the absence of other mitogens in 3T3 cells maintained in serum-free medium and (ii) a transient exposure of cultures to FDGF or PDGF causes a persistent stimulation of DNA synthesis. Either FDGF or PDGF enhances colony formation of non-transformed cells cultured in suspension in the presence of EGHF and serum. FDGF is not PDGF adsorbed by SV40-BHK cells from serum, since SV40-BHK cells plated and grown in the absence of serum still produce FDGF. In view of the similarities between PDGF and FDGF, we suggest that they may belong to the same family of growth factors. (Author abstract) (20 Refs)

44. FAILURE OF SOMATOSTATIN TO INFLUENCE EXPERIMENTAL TUMOR CELL GROWTH IN VIVO AND IN VITRO.

Lenzhofer R, Cerni C, Frohlich I, Prager J, Pointner H
1st Dept. Medicine, Lazarettgasse 14, A-1097 Vienna, Austria
Experientia; 37(9):1015-1017 1981

The influence of somatostatin on tumor cell growth was studied in vivo in mice (sarcoma 180 ascites tumor and Lewis lung tumor) and in vitro on nontransformed and polyoma-transformed cell lines. Four or 20 ug/100 g of cyclic somatostatin and 4 ug/100 g of linear protamine Zn-bound somatostatin were injected sc twice daily in the in vivo study. Cyclic somatostatin (1, 4 or 10 ug/ml) was added twice daily to the cell cultures. Somatostatin administration influenced neither the survival of animals nor the growth rate of cultured cell lines. (Author abstract) (9 Refs)

45. A NEW VIEW OF THE ETIOLOGY OF NASOPHARYNGEAL CARCINOMA.

Hirayama T, Ito Y
(c/o Ito) Dept. Microbiology, Faculty Medicine, Kyoto Univ., Kyoto 606, Japan
Prev Med; 10(5):614-622 1981

Epidemiological studies showed a high incidence of nasopharyngeal carcinoma (NPC) in southern regions of China. Also found in these regions are plants of the Euphorbiaceae family, including Croton tiglium (the source of croton oil) and other species used as herbal drugs. Interaction of herbal drugs and anti-Epstein-Barr virus (EBV) titers was noted. Use of the herbal drugs was related to nasal infection by microbes such as Fusobacterium. The combined effects of drug use and EBV induction were synergistic rather than additive. Fusobacteria in culture produced n-butyric acid, which had a low, but definite EBV-inducing activity in human lymphoblastoid P3HR-1 cells. Combined treatment with nanog concentrations of croton oil and n-butyrate produced marked increase in EBV antigens in EBV genome-carrying P3HR- 1 and Raji cells. Apparently EBV is induced and activated by the combination of bacterial fatty acid and herbal oils. It is suggested that the activated EBV then initiates steps resulting in NPC. (33 Refs)

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DNA TUMOR VIRUSES IN NON-PRIMATE SYSTEMS

SERIES CB11 No. 82/03

MARCH, 1982

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VIRUS ISOLATION/ STRUCTURE/ CHARACTERIZATION/ IDENTIFICATION

1. ANALYSIS OF THE DNA-TERMINAL PROTEIN FROM DIFFERENT SEROTYPES OF HUMAN ADENOVIRUS.

Rekosh D
Dept. Biochemistry, State Univ. New York at Buffalo, Buffalo, NY, 14214
J Virol; 40(1):329-333 1981

The DNA-terminal proteins from adenovirus Type 12, Type 3, and Type 5 were analyzed, after labeling in vitro with 125I by the chloramine T method, and were shown to be serotype specific. We also studied the kinetics of cleavage by alkali of the terminal protein-DNA linkage and showed that the half-time of cleavage with either 0.1 M NaOH or 0.5 M piperidine at 37 C is about 15 to 30 min. The substitution of the volatile base piperidine for NaOH in this procedure provided a useful

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tool for rapid analysis of the labeled protein. (Author abstract) (14 Refs)

2. B-LYMPHOTROPIC PAPOVAVIRUS (LPV) -- INFECTIONS OF MAN?

Brade L, Gissman L, Mueller-Lantzsch N, Zur Hausen H
Institut fur Virologie, Zentrum fur Hygiene, Hermann-Herderstrasse 11, 7800 Frieburg i. Br., W. Germany
Haematol Blood Transfus; 26:204-206 1981

Papovavirus-like particles that were first observed in supernatants of Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines from African green monkeys (AGM) were isolated and characterized. The host range for this virus consisted only of B-lymphoblasts from AGM and from one EBV-negative human cell line (line BJA-B), giving this virus the tentative designation lymphotropic papovavirus (LPV). LPV DNA is slightly smaller than SV40 DNA, weighing 3.2 x 10(6) daltons; it yields very different fragments than does SV40 DNA when these two DNA species are digested with Hae III enzyme. After greater than 1 yr of continuous passage in BJA-B cells, the LPV DNA became very heterogenous when examined by gel electrophoresis, apparently due to the accumulation of defective DNA molecules. LPV differs antigenically from all papovaviruses characterized so far. When human sera were tested for antibody to LPV, only about 10% of individuals less than 29 yr old were positive, whereas approx 30% of individuals greater than 30 yr old were positive. No disease-specific reactivity for LPV has so far been demonstrated, although some correlations between the presence of LPV antibodies in the sera and symptoms involving the lymphatic system have been noted. LPV may represent a new subgroup of papovaviruses that could have a role in proliferative diseases of the lymphatic system. (9 Refs)

3. CHARACTERIZATION OF A PARVOVIRUS STRAIN ISOLATED FROM HUMAN ADENOVIRUS TYPE 12.

Toth M, Lengyel A, Beladi I, Nasz I
Inst. Microbiology, Univ. Medical Sch., Dom ter 10, H-6720 Szeged, Hungary
Acta Microbiol Acad Sci Hung; 28(2):197-203 1981

Biological and physico-chemical properties of an isolated adenovirus-free parvovirus produced by heat treatment and propagated in HEp-2 cell culture are reported. The parvovirus strain PVSz was isolated from type 12 human adenovirus. PVSz multiplied without helper virus in HEp-2 cell cultures; type 12 and 18 adenoviruses potentiated the replication of PVSz. Gel filtration demonstrated two different hemagglutinins, one appearing in the void vol and the other close to the total vol. Ultracentrifugation in CsCl isopycnic gradient demonstrated that the low density fraction had a density of approx 1.25 g/cc. Rat sera contained different amounts of PVSz antibody, while pig sera contained negligible amounts. None of the sera from healthy humans inhibited the hemagglutination of the PVSz strain. When healthy laboratory animals were inoculated with PVSz, no cytopathic effect was observed, and except for a slight lymphocytic infiltration, no pathological alterations were found in the organs. Slight antibody production was observed in mice injected ip at the age of 2 mo. (15 Refs)

VIRUS ASSOCIATED CELL TRANSFORMATION AND HOST ALTERATION

4. BK VIRUS-TRANSFORMED INBRED HAMSTER BRAIN CELLS. I. STATUS OF THE VIRAL DNA AND THE ASSOCIATION OF BK VIRUS EARLY ANTIGENS WITH PURIFIED PLASMA MEMBRANES.

Beth E, Giraldo G, Schmidt-Ullrich R, Pater MM, Pater A, Di Mayorca G
Div. Viral Oncology, Instituto Nazionale Dei Tumori, 80131 Naples, Italy
J Virol; 40(1):276-284 1981

Inbred LSH hamster brain cells were transformed in vitro by the GS strain of BK virus (BKV), and transplantable tumors classified as undifferentiated glioblastomas were induced in the syngeneic host. The viral status in the transformed cells, designated LSH-BR-BK, was established. About 46 genome equivalents per cell of viral DNA was detected, with the majority of sequences in a free form. The transformed cells expressed large quantities of tumor (T) antigen as well as surface (S) antigen as demonstrated by indirect immunofluorescence. Sixty-three percent of tumor-bearing hamsters produced high-titer antibodies against T, whereas 3 of 14 (21%) hamsters also produced antibodies against the BKV-specific S antigen. Furthermore, the relatedness of BKV early gene products, including T, S, and tumor-specific transplantation antigen, was established by the production of a rabbit antiserum against highly purified plasma membranes of LSH-BR-BK cells and by the induction of a BKV-specific tumor-specific transplantation antigen response by these plasma membranes in the syngeneic host. (Author abstract) (47 Refs)

5. VIRAL GENE EXPRESSION IN POLYOMA VIRUS-TRANSFORMED RAT CELLS AND THEIR CURED REVERTANTS.

Fenton RG, Basilico C
Dept. Pathology, New York Univ. Sch. Medicine, New York, NY, 10016
J Virol; 40(1):150-163 1981

We have studied transcription of integrated viral DNA sequences in a variety of ts-a polyoma virus-transformed rat cells and cured revertants (which had undergone excision of variable amounts of integrated viral DNA) to characterize the structure of viral mRNA's produced in these lines under conditions in which integrated DNA is stable. Our results indicate that cells containing intact early region sequences, either in single-copy or tandem insertions, produce mRNA's indistinguishable from those observed early in lytic infection; sequences complementary to the polyoma late region were not transcribed from integrated viral DNA. Cured revertants no longer encoded full-length early mRNA's, but produced viral transcripts whose 3' ends mapped at an alternative early region polyadenylic acid attachment site at 99 map units or extended into flanking host sequences. The phenotype of these revertant cells correlated with the abundance of these transcripts, suggesting that the transforming function(s) of polyoma virus controls the cellular phenotype in a dose-dependent manner. Unexpected results were obtained from studies of cells containing tandem repeats of defective viral DNA in which the polyadenylic acid attachment signal at 25.8 map units and surrounding sequences were deleted. In these cases, polyadenylated mRNA's were observed that contained sequences complementary to the early strand of the polyoma late region. These mRNA's (some larger than 8 kilobases) originated at the viral

early promoter, extended into the late region, and continued into the early region of the contiguous repeat in the tandem. The multimeric mRNA's produced contained defective early regions in tandem with late region sequences. S1 analysis indicated that whereas the 5' early region sequences of readthrough transcripts were spliced in the usual manner, internal early region repeats were either unspliced or used only one of the small early region splices. When deletions in the viral DNA began 40 base pairs past the AAUAAA sequence at 25.8 map units, no readthrough transcripts were observed. This suggests that sequences nearby the AAUAAA sequence at 26 map units may control transcription termination of the polyoma early region. (Author abstract) (34 Refs)

6. TUMORIGENIC ACTIVITY OF CLONED POLYOMA VIRUS DNA IN NEWBORN RATS.

Gelinas C, Bouchard L, Bastin M

Departement de Microbiologie, Centre Hospitalier Universitaire, Universite de Sherbrooke, Sherbrooke, Quebec J1H 5N4, Canada

Experientia; 37(10):1074-1075 1981

The proximal portion of the polyoma virus early region as well as the complete viral genome were cloned in pBR322. Recombinant plasmids induced tumors in newborn rats but only after linearization of the DNA by various restriction endonucleases. (Author abstract) (11 Refs)

7. THE EXPRESSION OF PAPOVAVIRUS TUMOR ANTIGENS IN EMBRYONAL CARCINOMA CELLS (MEETING ABSTRACT).

Levine AJ, Sekikawa K

Dept. of Microbiology, State University of New York at Stony Brook School of Medicine, Stony Brook, NY, 11794

Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, January 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

Polyoma virus undergoes a productive infection and SV40 expresses its early proteins in most differentiated mouse cells. Neither virus produces its early proteins in murine embryonal carcinoma cells (EC cells). When these EC cells differentiate then these cells produce infectious polyoma virus or can be transformed by SV40 and express early viral proteins. Thus an investigation of the mechanisms involved in the papovavirus host range restriction in EC cells may prove useful in exploring the cellular control mechanisms operative during development changes. In F9 EC cells infected with SV40, low levels of viral tran scriptional intermediates and viral RNA have been detected (1) but the SV40 RNA is not spliced into large and small T-antigen (the viral early proteins) m-RNA's. When F9 EC cells were stimulated to differentiate into endoderm, then SV40 m-RNA's were properly processed and viral proteins were synthesized. Polyoma host range mutants that now replicate in F9 EC cells have been isolated. These mutant viruses produce immunologically detectable levels of the viral tumor antigens of the same mol wt as the wild type virus in differentiated cells. Two independently derived host range mutants contain the same single base alteration (AT-GC) and a 33 and 67 base pair tandem duplication of those viral DNA sequences containing the point mutation. This duplication is located at 69 map units of the polyoma genome on the late gene side of the origin of viral DNA replication (at 70.5 map units). This type of mutation suggests several models to explain the polyoma host range restriction in EC cells. (3 Refs)

8. PHENOTYPIC CHANGE OF AN SV40-TRANSFORMED MOUSE MACROPHAGE LINE, BB-W-531-2, INDUCED BY DIFFERENT CULTURAL METHODS.

Takayama H, Tanigawa T, Takagi A

Dept. Bacteriology, Tottori Univ. Sch. Medicine, Yonago, Tottori 683, Japan

Microbiol Immunol; 25(8):827-835 1981

The effect of culture methods on the characteristics of Simian virus 40 (SV40)-transformed macrophages, BB-W-531-2, established from BALB/cAN mouse bone marrow cells was studied. Results indicated that cell growth and differentiated cell properties of clonal macrophages (BB-W-531-2 line) are controlled by culture methods. The cells passaged by the first method showed a reversible transition between the macrophage and non-macrophage states depending upon cell density. The cell passaged by the second method retained their macrophage properties throughout the culture and grew very slowly. The cells passaged by the third method did not show density-dependent transition. Macrophage properties, such as immune phagocytosis, seemed to be induced by diffusible factor(s) produced by cells possessing such properties. The fact that several properties of BB-W-531-2 cells can be controlled by the culture methods described above suggested the potential value of such control in studies of gene expression regulation. BB-W-531-2 cell growth was correlated with the intensity of SV40 T-antigen and seemed to be suppressed in poor-spreading conditions such as confluent cell density or cultivation on bacteriologic dishes. The development of macrophage properties in cultures of confluent density suggested that both density-dependent grow inhibition and diffusible factors are probably required for induction of macrophage properties. (20 Refs)

9. ANCHORAGE-INDEPENDENT GROWTH OF NORMAL CALF LENS EPITHELIAL CELLS AND EFFECT OF SV40 TRANSFORMATION ON THEIR GROWTH PROPERTIES.

Shearer M, Taylor-Papadimitriou J

(c/o Taylor-Papadimitriou) P.O. Box 732, Lincoln's Inn Fields, London, WC2A 3PX, England

J Cell Physiol; 108(3):385-391 1981

The growth properties of normal calf lens epithelium and of Simian virus 40 transformants of these cells (SV LE.1 cells) were studied in relation to malignant change in epithelial cells. Growth of normal and SV LE.1 cells was obtained with a very low level of added mitogens. In low serum, the growth of SV LE.1 and normal lens cells appeared to be similar, although a higher cell density was reached by transformed cells at higher serum levels. In a medium containing 10% serum, SV LE.1 cells proliferated faster and achieved a higher density than did normal cells. Thus viral transformation decreased doubling time and increased final cell density when subject to strong mitogenic stimulus (high serum). Both normal and SV LE.1 cells formed mutlilayers when grown on plastic, the final cell density reached in 10% serum being 3.2 x 10(4) and 1.4 x 10(5)/square cm, respectively. It appeared that the constant number of attached cells in cultures of normal and SV LE.1 cells was maintained by cells detaching and being replaced by proliferating cells. There was considerable variation in the size of normal cells, but most of them were larger than the SV LE.1 cells. Of normal lens cells suspended in agar, 40-50% formed colonies that reached a size of 0.1-0.2 mm after 5 days in culture. The high cloning efficiency ruled out the possibility that colonies originated by aggregation. Growth from single cells was demonstrated. SV LE.1 cells formed colonies with a somewhat lower efficiency than lens cells, but the size of these colonies was larger. The results indicated that calf lens epithe-

lial cells exhibit growth properties in vitro that have usually been associated with virally transformed fibroblasts. (23 Refs)

10. ANTISERUM TO SV40 T ANTIGENS FROM A RABBIT INOCULATED WITH AUTOCHTHONOUS CELLS TRANSFORMED IN VITRO BY SV40.

Tamura H, Shimura H, Kimura G
First Dept. Surgery, Tottori Univ. Sch. Medicine, Nishimachi 36-1, Yonago 683, Japan
Gan; 72(5):769-771 1981

A morphologically transformed cell line was established after infection with SV40 of the primary culture derived from a kidney removed surgically from a rabbit. The transformed cells were free of detectable infectious SV40 particles but produced them upon fusion with permissive monkey cells, and produced SV40-specific large and small T antigens. Repeated sc inoculations of the transformed cells into the animal from which the parental kidney cells had been derived failed to result in tumor formation but raised a substantial level of antibodies to the SV40-specific large and small T antigens as well as to the 58,000 dalton mol wt cellular protein (middle T antigen) (Author abstract) (10 Refs)

11. CARCINOGEN-MEDIATED AMPLIFICATION OF VIRAL DNA SEQUENCES IN SIMIAN VIRUS 40-TRANSFORMED CHINESE HAMSTER EMBRYO CELLS.

Lavi S
Dept. Virology, Weizmann Inst. Science, Rehovot, Israel
Proc Natl Acad Sci USA; 78(10):6144-6148 1981

Exposure of simian virus 40 (SV40)-transformed Chinese hamster embryo cells to various chemical and physical carcinogens induced SV40 DNA synthesis. Although the carcinogen-mediated amplification of SV40 DNA is regulated by the viral A gene, the induction of viral DNA synthesis does not result in the rescue of infectious virus or the formation of complete viral DNA molecules. Instead, a heterogenous collection of DNA molecules containing SV40 sequences was generated by treatment with 7,12-dimethylbenz[a]anthracene. Restriction enzyme analysis of the amplified DNA molecules in the Hirt supernatant showed that not all sequences in the integrated SV40 inserts are present. The possibility that amplification of SV40 sequences is a reflection of a general gene-amplification phenomenon mediated by carcinogens is discussed. (Author abstract) (29 Refs)

12. TRANSCRIPTION OF THE SIMIAN VIRUS 40 GENOME IN DNA-TRANSFORMED MURINE TERATOCARCINOMA STEM CELLS.

Linnenbach A, Huebner K, Croce CM
Wistar Inst. Anatomy and Biology, 36th St. at Spruce, Philadelphia, PA, 19104
Proc Natl Acad Sci USA; 78(10):6386-6390 1981

To study the molecular basis for lack of expression of the simian virus 40 (SV40) early region genes in murine teratocarcinoma-derived stem cells, we introduced a recombinant plasmid consisting of pBR322 linked to the herpes simplex virus Type 1 thymidine kinase gene and SV40 genome into thymidine kinase-deficient F9 stem cells. The resulting stem cell clone, 12-1, and a retinoic acid-induced differentiated daughter cell clone, 12-1a, each contain one copy per cell of the entire recombinant plasmid integrated into the cellular genome through a site on the pBR322 genome. Restriction endonuclease analyses indicate that there is no difference in integration site or organization of the three component parts of the plasmid genome within cellular DNA of stem and differentiated cells; yet the differentiated cells, 12-1a, express SV40 large tumor antigen whereas the stem cells, 12-1, do not. Both stem and differentiated cells produce two size classes of polyadenylated RNA, 2,900 and 2,600 bases in length, homologous to the early region of the SV40 genome, detectable by RNA blotting analysis. S1 nuclease analysis of the SV40 transcripts present in stem and differentiated cells indicate that the SV40 mRNAs were identically spliced in the two cell types, in a manner consistent with that observed for spliced large and small tumor antigen mRNAs in SV40-infected monkey kidney cells. Thus, the failure of 12-1 teratocarcinoma stem cells, containing an integrated SV40 genome , to express SV40 tumor antigen is not due to a lack of transcription of the SV40 early region or to an inability to splice primary transcripts. (Author abstract) (44 Refs)

13. PHOSPHORYLATION PATTERNS OF TUMOR ANTIGENS IN CELLS LYTICALLY INFECTED OR TRANSFORMED BY SIMIAN VIRUS 40.

Van Roy F, Fransen L, Fiers W
Lab. Molecular Biology, State Univ. Ghent, B-9000 Ghent, Belgium
J Virol; 40(1):28-44 1981

The phosphorylation sites of simian virus 40 (SV40) large tumor (T) antigens have been analyzed by partial proteolysis peptide mapping and phosphoamino acid analysis of the resulting products. At least four sites were found to be phosphorylated. An amino-terminal part of the molecule contained both phosphoserine and phosphothreonine. One phosphothreonine residue was located in the proline-rich carboxy-terminal end of the molecule, either at position 701 or at position 708. The mutant dl1265, which is defective in adenovirus helper function, lacked this phosphorylation site. In addition, the carboxy-terminal part of the molecule contained phosphoserine at a more central position. T-antigen-associated proteins of SV40-transformed cells (nonviral T; 51,000 to 55,000 daltons) also contained multiple phosphorylation sites involving at least two serine residues in mouse antigens and an additional threonine residue in rat, human, and monkey antigens. The latter residue and at least one phosphoserine residue were located near one terminus of the human NVT molecule. We did not find any evidence for phosphorylation of tyrosine residues in any of the multiple species of either large T or nonviral T molecules. Several forms of large T antigens were extracted from both SV40-transformed and SV40-infected permissive and nonpermissive cells, and their phosphorylation patterns were compared. No evidence was found for a different phosphorylation pattern of T antigens in transformed cells. (Author abstract) (62 Refs)

14. SV40 RECOMBINANT MOLECULES EXPRESS THE GENE ENCODING P21 TRANSFORMING PROTEIN OF HARVEY MURINE SARCOMA VIRUS.

Gruss P, Ellis RW, Shih TY, Konig M, Scolnick EM, Khoury G
Lab. Molecular Virology, NCI, Bethesda, MD, 20205
Nature; 293(5832):486-488 1981

Construction of the simian virus 40 (SV40)-Harvey murine sarcoma virus (Ha-MuSV) recombinants is described. The presence of a Ha-MuSV promoter element preceding the gene encoding p21 was shown. The new promoter element was found to be active not only in cells infected with recombinant but also in cells transformed with Ha-MuSV. (32 Refs)

15. ENHANCEMENT OF ANTIBODY-COMPLEMENT CYTOTOXICITY AGAINST VIRUS-TRANSFORMED HAMSTER PARA-7 CELLS TREATED WITH HEAT AND MICROWAVE RADIATION.

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Dept. Electrical Engineering, Univ. Illinois at Urbana-Champaign, Urbana, IL, 61801
Radiat Res; 88(1):96-107 1981

Effects of both water-bath heating and microwave exposure on the sensitivity of Simian virus(SV) 40-transformed hamster cells (PARA 7) to cytotoxic effects of antibody and complement are reported. The PARA 7 tumor cell line was obtained from transformation of LSH hamster embryo fibroblasts into fibrosarcoma. Three ml PARA-7 cell suspensions were harvested, resuspended and irradiated in liquid scintillation vials with microwaves (100 milliWatts(Ws)/square cm; 105 Ws/kg). Antibody-complement (Ab-C) cytotoxicity was assayed with 51Cr release and colony-formation (CF). Progressive loss of cell viability was observed when cells were heated for 30 min or more at 37-43 C. The heated cells showed increased sensitivity to Ab-C cytotoxicity when antibodies directed against whole cell membranes or cell membrane-associated SV40 tumor-specific antigen was used. PARA 7 cells exposed to microwaves under the same variable temperatures show similar loss of cell viability and increased sensitivity to Ab-C cytotoxicity, but under constant temperature (37 C), based on both the 51Cr release and CF assays, exposure to microwaves did not result in significant loss in viabilty or sensitivity to Ab-C. The results suggested that the effects of microwave exposure are thermal in origin. The data also showed that the loss of cell viability and sensitization to Ab-C cytotoxicity following water-bath heating or microwave exposure are not significantly different if the temperature is held constant. (28 Refs)

16. INHIBITION OF THE PLATELET-AGGREGATING ACTIVITY OF TWO HUMAN ADENOCARCINOMAS OF THE COLON AND AN ANAPLASTIC MURINE TUMOR WITH A SPECIFIC THROMBIN INHIBITOR, DANSYLARGININE N-(3-ETHYL-1,5-PENTANEDIYL)AMIDE.

Pearlstein E, Ambrogio C, Gasic G, Karpatkin S
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Cancer Res; 41(11,part1):4535-4539 1981

Platelets are required for certain experimental metastases. Several lines of animal tumor cells aggregate platelets in vitro and in vivo. Previous studies with one of these lines, an SV40-transformed 3T3 mouse fibroblast (SV3T3) have revealed that the platelet-aggregating material is an extractable membrane-associated sialolipoprotein which requires divalent cation, complement, and a heat-stable plasma component for activity. Little information is available on the interaction of human tumors with platelets. We now report on the ability of two human adenocarcinomas of the colon (LoVo and HCT-8) and an anaplastic mouse tumor (Hut-20) to aggregate platelets by a different mechanism, the generation of thrombin. These spontaneous cell lines aggregate human or rabbit platelet-rich plasma after a 1-to 2-min lag period. This is often followed by a visible clot. Unlike SV3T3 cells, aggregation by LoVo, HCT-8, and Hut-20 cells i s not inhibited by neuraminidase, trypsin, or cobra venom factor. These three cell lines markedly shorten the recalcification time of citrated plasma, whereas SV3T3 cells do not. Phospholipase A2 treatment inhibits the shortening of the recalcification time for the three tumors; this parallels its inhibitory effect on platelet aggregation. LoVo, HCT-8, and Hut-20 cells generate thrombin via the 'tissue factor' coagulation pathway (using coagulation factor-deficient substrates). Dansylarginine-N-(3-ethyl-1,5-pentanediyl)amide, a highly specific, potent antithrombin antagonist, inhibits LoVo-, HCT-8-, and Hut-20-induced platelet aggregation at 4 to 15 uM, whereas its effect on SV3T3 cells is negligible. If platelets are required for certain human tumor metastases, dansylarginine-N-(3-ethyl-1,5-pentanediyl)amide, or other antithrombin agents, may prove to be valuable therapeutic agents. (Author abstract) (37 Refs)

17. TARGETED KILLING OF CULTURED CELLS BY RECEPTOR-DEPENDENT PHOTOSENSITIZATION.

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Proc Natl Acad Sci USA; 78(9,part2):5717-5721 1981

This paper describes a method, designated 'receptor-dependent photosensitization', by which the receptor-mediated endocytosis of low density lipoprotein (LDL) can be used to deliver a photosensitizing agent, pyrene, to cultured human and animal cells. The hydrophobic core of LDL is extracted and replaced with pyrene covalently coupled to cholesteryl oleate. This reconstituted LDL enters cells in significant amounts only when the cells express LDL receptors, resulting in the accumulation of pyrene cholesteryl oleate within lysosomes. Subsequent exposure of the cells to UV light leads to cell death. Cells killed by this technique include normal and simian virus 40-transformed human fibroblasts, human A-431 epidermal carcinoma cells, Chinese hamster ovary cells, and mouse L cells, all of which express LDL receptors. Mutant fibroblasts from a patient with homozygous familial hypercholesterolemia, which lack LDL receptors, do not take up significant amounts of the pyrene-containing LDL and are not killed by subsequent exposure to light. The current experiments establish the feasibility of receptor-dependent photosensitization as an efficient and selective method for killing cultured human and animal cells. (Author abstract) (24 Refs)

18. EPISOMAL SIMIAN VIRUS 40 GENOMES IN HUMAN BRAIN TUMORS.

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Proc Natl Acad Sci USA; 78(10):6446-6450 1981

Eight out of 35 human intracranial tumors were shown by restriction enzyme analysis to contain unintegrated simian virus 40 (SV40) DNA molecules. The relative amount of viral DNA was estimated to be the equivalent of one viral genome within every 10th to 20th cell. No infectious virus was detected in tissue cultures established from the tumors. From only one tumor was it possible to rescue, by cell fusion, infectious SV40 displaying wild-type properties. In those cases that permitted a more detailed analysis, the restriction enzyme cleavage patterns appeared to correspond to the wild-type patterns with one exception, in which the SV40 episomes displayed a deletion of approx 70 base pairs close to the origin of DNA replication. From one tumor, the SV40 genomes were transferred into permissive CV-1 monkey cells by transfection with the total tumor DNA. Despite their persistence as episomes no infectious virus was produced. Furthermore, no viral antigens were detectable, although the SV40 messengers for the small and the large tumor antigens were present. These cells had, however, acquired the ability to form colonies in lower concentrations of

serum. Thus this report provides, by restriction enzyme analysis, direct evidence for the presence of SV40 DNA in human tumors. (Author abstract) (34 Refs)

19. ON THE RELATIONS BETWEEN VIRUS-INDUCED MUTATION AND NEOPLASTIC TRANSFORMATION IN RODENT AND HUMAN CELLS (MEETING ABSTRACT).

Theile M, Waehlte H, Luebbe L, Krause H, Scherneck S, Strauss M, Geissler E
Central Institute of Molecular Biology, Academy of Sciences of the GDR, Berlin-Buch, E. Germany
Eleventh Annual Meeting of the European Environmental Mutagen Society, July 6-11, 1981, Budapest, Hungary The Society; 1981

Simian Virus 40 and other oncogenic DNA viruses, in addition to their transforming capacity, show the ability to induce gene mutations in the infected cells although it is not known to what extent these two effects depend on each other. Analyzing virus-induced mutational alterations the following results were obtained: 1) In SV40 transformed cells the frequencies of both forward mutations and reversions are increased compared to those in corresponding non-transformed cells and, vice versa. SV40-induced mutant clones frequently seem to be transformed. Moreover both mutation and transformation depend on the virus dose. 2) Further parallels exist between the mutagenic and transforming effects in hamster cells infected with virus (DNA) as well as after combined treatment by virus and chemical mutagens. The results can be explained by the assumption that both kinds of cellular alterations which are initiated by viral infection have some essential steps in common. (no Refs)

20. NUCLEAR MAGNETIC RESONANCE SPIN-LATTICE TIMES OF NORMAL AND TRANSFORMED CULTURED MAMMALIAN CELLS AND OF NORMAL AND NEOPLASTIC ANIMAL TISSUES.

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Whiteshell Nucler Res. Establishment, Atomic Energy of Canada, Pinawa, Manitoba, Canada
Physiol Chem Phys; 13(3):251-258 1981

The proton spin-lattice relaxation time (T1) was measured in normal and neoplastic tissues of mice and in normal and transformed cultured cells; the water content of various tissues was compared with the T1 values; and the effects on T1 of various perturbations was investigated in tissue and cell samples. Tumor tissue gave much longer T1 values (p less than 0.01) than did healthy tissue from either a tumor-bearing or healthy mouse; it was irrelevant whether the mouse had or had not been infected with Salmonella typhimurium, showing that the observed increase in T1 was not due to an increase in cellular immunity. The T1 values of normal 3T3 cells and of SV40-transformed cells were the same, which implies that the changes in T1 in neoplastic tissue may be due to interaction of the neoplasm with its physiological environment rather than to any intrinsic property of cancer cells. The correlation coefficient was 0.985 between T1 and the percent water content of each of five rat tissues; tissues with higher water content consistently exhibited longer T1s. The addition of water to rat tissues by glass capillary resulted in longer T1 values. When tissue samples were sealed in nuclear magnetic resonance tubes (to keep the water content constant) and subjected to boiling, freezing ,or storage for various times, the T1 values were altered. These results indicate that T1 values may be influenced by both cellular water content and by macromolecular conformation. (33 Refs)

21. IDENTIFICATION OF A VIRUS-SPECIFIC POLYPEPTIDE ASSOCIATED WITH A TRANSFORMING FRAGMENT (BGLII-N) OF HERPES SIMPLEX VIRUS TYPE 2 DNA.

Docherty JJ, Subak-Sharpe JH, Preston CM
Microbiology Program, Pennsylvania State Univ., University Park, PA, 16802
J Virol; 40(1):126-132 1981

The BglI N fragment of herpes simplex virus Type 2 (HSV-2) DNA (approx 0.58 to 0.63 map unit) was examined for encoded products. Using plasmid pGZ59, which consists of BglII-N cloned in pAT153, in conjunction with hybrid-arrested translation, mRNA selection, and in vitro protein synthesis, we found that the major translated product of this region has an approx mol wt of 37,800. By further mapping, coding sequences for this polypeptide were located within the region of BglII-N representing approx 0.58 to 0.61 genome map unit. To demonstrate immunological specificity, we used staphylococcal A protein immunoprecipitation with rabbit anti-HSV-1 or HSV-2 sera and antigens from HSV-1 or HSV-2 total mRNA translated in vitro and BglII-N-selected mRNA. The results show that the 37,800-dalton polypeptide has HSV-2 immunological specificity, as it is precipitated with anti-HSV-2 sera but not with anti-HSV-1 or control sera. (Author abstract) (20 Refs)

22. SEQUENCE COMPLEXITY OF CIRCULAR EPSTEIN-BARR VIRUS DNA IN TRANSFORMED CELLS.

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J Virol; 40(1):11-19 1981

A simplified procedure, based on several methods previously used to isolate circular DNA molecules from bacteria, was devised for the preparation of covalently closed circular viral DNA molecules from large quantities of lymphocytes transformed by Epstein-Barr virus. The protocol can be applied both to virus nonproducer lines and to lines containing cells activated to virus production. Sufficient amounts of highly purified viral DNA of intracellular origin were obtained from B95-8 and Raji cells to allow direct visual analysis of their sequence complexities after cleavage with EcoRI and separation of fragments by gel electrophoresis. No major differences in complexity were observed between circular DNA and linear virion DNA from B95-8 cells. The fragment patterns observed in this fashion agree well with those detected by conventional blotting and hybridization methods. The procedure can also be used as an analytical method to assay for small amounts of circular Epstein-Barr virus DNA molecules in other transformed cells. In this connection, no circular Epstein-Barr virus DNA was detected in Namalva Cells. (Author abstract) (32 Refs)

23. FOCUS FORMATION AND NEOPLASTIC TRANSFORMATION BY HERPES SIMPLEX VIRUS TYPE 2 INACTIVATED INTRACELLULARLY BY 5-BROMO-2'-DEOXYURIDINE AND NEAR UV LIGHT.

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Dept. Comparative Medicine and Biochemistry, Johns Hopkins Medical Inst., Baltimore, MD, 21205
J Virol; 40(1):289-300 1981

The induction of focus formation in low serum and of neoplastic transformation of Syrian hamster embryo cells was examined after the expression of herpes simplex virus Type 2

functions. Syrian hamster embryo cells infected at a high multiplicity (5 PFU/cell) with 5-bromo-2'-deoxyuridine-labeled herpes simplex virus Type 2 (11% substitution of thymidine residues) were exposed to near UV light irradiation at various times postinfection. this procedure specifically inactivated the viral genome, while having little, if any, effect on the unlabeled cellular DNA. Focus formation in 1% serum and neoplastic transformation were observed in cells exposed to virus inactivated before infection, but the frequency was enhanced (15-to 27-fold) in cells in which the virus was inactivated at 4 to 8 hr postinfection. Only 2 of 45 independently isolated foci were capable of establishing tumorigenic lines. The established lines exhibited phenotypic alterations characteristic of a transformed state, including reduced serum requirement, anchorage-independent growth and tumorigenicity. They retained viral DNA sequences and, even at relatively late passage, expressed viral antigens, including ICP 10. (Author abstract) (44 Refs)

24. IGG-FC-BINDING RECEPTORS IN CELLS ABORTIVELY INFECTED, OR TRANSFORMED, BY HUMAN CYTOMEGALOVIRUS.

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Acta Microbiol Acad Sci Hung; 28(2):157-164 1981

IgG-Fc-binding membrane and cytoplasmic receptors were investigated in human embryonic fibroblast and hamster embryonic fibroblast cultures prepared, respectively, from human abortion material and Syrian golden hamster embryos, and also in cells transformed by human cytomegalovirus (HCMV). Cytoplasmic receptors were formed in abortively infected cells and similar receptors were formed on the cell surface of a low proportion (12-15%) of the cells infected either productively (human cells) or abortively (hamster cells). In the presence of cytosine arabinoside hydrochloride (ara-C), the receptors were formed in both the cytoplasm and cell membrane more readily than without ara-C. Cytoplasmic IgG-Fc receptors had formed in a very low proportion (2%) of the human cells and in a much higher proportion (65%) of the hamster cells while DNA synthesis was inhibited. IgG-Fc-binding receptors were transiently present in the cytoplasm and on the surface of cells being abortively infected by HCMV. The receptors were also present in cells transformed in vitro by HCMV (87-TRH-5 and CX-90-3B), as well as in cells of lines TSC-1 and TSC-2 from tumors induced by these cells. Thus, the indirect immunofluorescence test performed with sera containing no HCMV antibodies may give a positive reaction in the cytoplasm and/or cell membrane of the transformed cells. (18 Refs)

25. CHROMOSOME 14 MARKERS IN TWO EPSTEIN-BARR VIRUS (EBV)-TRANSFORMED LYMPHOBLASTOID CELL LINES OF NORMAL ORIGIN DIFFER FROM THE BURKITT LYMPHOMA (BL)-ASSOCIATED 14Q+ MARKER.

Manolov G, Manolova Y, Klein G, Levan A, Kieler J
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Cancer Genet Cytogenet; 4(2):179-184 1981

Two among 10 Epstein-Barr Virus (EBV)-transformed lymphoblastoid cell lines contained a 14q+ marker in a low frequency of the cells (2 and 9%). By means of 'mesome-prosome' analysis of the G-band patterns of these markers; it was established that the additional chromosome segments of these two 14q+ markers came from chromosomes 3 and 5, respectively, and not from chromosome 8 as in the 14q+ marker of Burkitt lymphoma. Chromosome 8 was not involved at all in any changes in the 10 lymphoblastoid cell lines studied. (Author abstract) (19 Refs)

26. DIPLOID HUMAN LYMPHOBLASTOID AND BURKITT LYMPHOMA CELL LINES: SUSCEPTIBILITY TO MURINE NK CELLS AND HETEROTRANSPLANTATION TO NUDE MICE.

McCormick KJ, Giovanella BC, Klein G, Nilsson K, Stehlin JS
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Int J Cancer; 28(4):455-458 1981

Human lymphoid cell lines which had been classified on the basis of studies on clonality and morphological, chromosomal and functional parameters as lymphoblastoid cell lines (LCL) of presumed non-neoplastic origin and Burkitt lymphoma (BL) lines of proven malignant origin, were tested for susceptibility to natural killer (NK) cells obtained from the spleens of athymic nude mice. The 20 lines included normal diploid LCL and aneuploid BL lines. All cells carried the Epstein-Barr virus (EBV) genome. In addition, two EBV-negative BL lines were tested. The pronase-induced release of 14C-DNA from 14C-thymidine-labeled target cells was used to assess the sensitivity of the cell lines to NK activity. When attempts were made to correlate the growth of the EBV-positive LCL and the EBV-positive BL cell lines in the sc space of adult nude mice with their susceptibility to NK cells, no significant correlation was observed. The EBV-negative BL cell line, Ramos, however, could be transplanted subcutaneously in nude mice and was more resistant to NK activity than was the EBV-negative BL cell line, BJAB, which cannot be transplanted subcutaneously. Growth of heterotransplanted EBV-converted cell lines in the sc space of adult nude mice may be influenced by immune effectors other than NK cells. (Author abstract) (23 Refs)

27. STEROIDS INHIBIT TUMOR PROMOTING AGENT INDUCED EPSTEIN-BARR VIRUS EARLY ANTIGENS IN RAJI CELLS.

Sundar SK, Ablashi DV, Armstrong GR, Zipkin M, Faggioni A, Levine PH
Div. Cancer Cause and Prevention, NCI, Bethesda, MD, 20205
Int J Cancer; 28(4):503-507 1981

Four steroids and one protease inhibitor were evaluated for their effects on 12-O-tetradecanoyl phorbol 13-acetate (TPA)-induced and Epstein-Barr virus-induced early antigens (EBV-EA) in Raji cells. Continuous treatment with dexamethasone, prednisolone, hydrocortisone and cortisone inhibited TPA-induced EBV-EA to varying degrees, but the protease inhibitor N-alpha-p-tosyl L-lysine chloromethyl ketone-HCl (TLCK) had no significant effect. None of the agents tested inhibited EBV-induced EA. In addition, the effect of the steroids was reversible since the removal of these agents resulted in recovery of the percentage of EBV-EA-positive cells in TPA-treated cultures. These results were in agreement with the in vivo experiments of other investigators, who demonstrated inhibition of tumor promotion with steroids. Since TLCK failed to inhibit TPA-induced EA, it is unlikely that induction of EA by TPA is the result of production of proteases. (Author abstract) (36 Refs)

28. LYMPHOPROLIFERATION AND HETEROTRANSPLANTATION IN NUDE MICE: TUMOR CELLS IN HODGKIN'S DISEASE.

Diehl V, Kirchner HH, Schaadt M, Fonatsch C, Stein H
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Haematol Blood Transfus; 26:229-234 1981

Histologically proven Hodgkin's disease (HD) derived material was used to establish four long term cultures. These in vitro cells had morphologic, cytochemical and cytogenetic features of their in vivo ancestors. Two of these cultures were obtained from pleural effusions of HD nodular sclerosing type, Stage IV B (diagnosed, one in 1972 and another in 1976). They shared these same features: non-B-T lymphocytes, non-macrophages, non myeloid cells, Epstein-Barr virus genome negative, monoclonality, multiple numerical and structural chromosome aberrations, and tumor formation after intracranial xenotransplantation in nude mice. After sc inoculation both lines failed to induce tumors. When one of them was embedded in a fibrin clot prior to transplantation, infiltrative tumor growth resulted after sc inoculation. Morphology of the two cell lines was very similar to Hodgkin (H) and Sternberg-Reed (SR) cells. Biological and immunological characterization of the two lines showed identical pattern observed in freshly prepared H and SR cells. They resemble very closely cultured H cells described by Kaplan. (14 Refs)

29. EPISOMAL AND NONEPISOMAL HERPESVIRUS DNA IN LYMPHOID TUMOR CELL LINES.

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Haematol Blood Transfus; 26:197-203 1981

Experiments were carried out on two oncogenic viruses of New World primates Herpesvirus saimiri and Herpesvirus ateles, to study the persistence of the viral DNA in host cells and the transformation of host cells by these viruses. The complete viral genomes are linear duplex DNA that contain a unique segment of L-DNA and two terminal segments of H-DNA. Transformed lymphoid tumor cells contain substantial amounts of viral L- and H-DNA, apparently as nonintegrated circular superhelical episomal DNA; they also yield a viral DNA fraction (after digestion with restriction endonucleases) that is not found in the episomal viral DNA, but the exact nature of this fraction is not yet known. The resistance of viral DNA to certain restriction endonucleases and the measured buoyant density of this DNA in cesium chloride gradients suggests that the viral DNA (both episomal and nonepisomal) is extensively methylated in nonproducer cell lines. This methylation can be reduced by conventional inhibitors of DNA methylation and by the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate. The significance of the inhibition of methylation by this tumor promoter is not known. (17 Refs)

30. METASTATIC PROPERTIES AND CELL SURFACE GLYCOPROTEINS OF A HAMSTER TUMOUR OF HSV AETIOLOGY AND ITS TWO METASTATIC SUBLINES (MEETING ABSTRACT).

Rees RC, Walker JR
Department of Virology, The University of Sheffield Medical School, Sheffield, England
Br J Cancer; 43(5):722 1981

The acceptance of a realistic animal model for human malignancy requires that the tumor should not be highly antigenic or encapsulated, but that cells should spontaneously metastasize to sites distant from the primary solid mass. Two such metastatic sublines have been isolated from a hamster tumor of herpesvirus hominis-2 etiology (333-2-26). These metA and MetB lines were established by in vivo sc transplantation of lung metastatic foci which occurred at low frequency in hamster after resection of the primary tumor. Both lines were considerably more metastatic than the parent; hamster mortality due to metastasis was typically 100% 40 days after tumor resection compared with 20% in the parent line. The efficiency of plating in soft agar of the parental and metA and metB lines was assessed; no correlation between anchorage-independent growth and metastatic potential was seen. In addition, the cell-surface glycoprotein profile was anlayzed by polyacrylamide gel electrophoresis after labelling by pretreatment with galactose oxidase or periodate and borotritiide reduction. Development of metastatic potential was correlated with an increase in high mol wt species of surface glycoproteins possessing a terminal neuraminic acid and subterminal galactose residue. (no Refs)

31. CARCINOGENIC EFFECT OF HERPES SIMPLEX VIRUSES TYPES 1 AND 2 (HSV-1 AND 2) IN THE MOUSE (MEETING ABSTRACT).

Wentz WB, Reagan JW, Heggie AD, Fu YS, Anthony DD
No affiliation given
J Pediatr; 98(6):1015 1981

The objective of this study was to determine if prolonged exposure of the mouse cervix to inactivated HSV-1 and HSV-2 would result in induction of cervical cancer. C57 mice were exposed to formalin-inactivated HSV-1 or HSV-2, or to UV-inactivated HSV-2 by insertion of virus-saturated cotton pledgets into the vagina five x wk for 80 wk. Control mice were exposed to control fluids prepared in the same way as virus stocks except for the absence of virus. Cervical cancer was detected in 24 to 60% and dysplasia in 18 to 66% of virus-exposed mice. All controls were normal. The frequency of cancer in the mice exposed to UV-inactivated HSV-2 was twice that associated with formalin-inactivated virus. In addition to cervical cancers, endometrial cancers were detected in 7 to 28% of virus-exposed mice, but not in controls. To determine if the oncogenic effect of HSV was specific for the genital tract in mice, formalin-inactivated HSV-1 or HSV-2 was instilled into the nasopharynx of mice for 12 to 32 wk. No premalignant or malignant lesions of the naso- or oropharynx or lower respiratory tract were detected. This study shows that, in the mouse, (1) prolonged exposure of the female genital tract to inactivated HSV-1 or HSV-2 induces cervical dysplasia and carci noma. (2) UV-inactivated HSV-2 is more carcinogenic to the cervix than is formalin-inactivated virus, and (3) exposure to inactivated HSV-1 or HSV-2 also induces endometrial carcinoma but with lower frequency than cervical carcinoma. (no Refs)

VIRUS REPLICATION AND EXPRESSION

32. SIMIAN VIRUS 40 EARLY MRNA'S CONTAIN MULTIPLE 5' TERMINI UPSTREAM AND DOWNSTREAM FROM A HOGNESS-GOLDBERG SEQUENCE; A SHIFT IN 5' TERMINI DURING THE LYTIC CYCLE IS MEDIATED BY LARGE T ANTIGEN.

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J Virol; 40(1):224-240 1981

We have used primer-directed synthesis, separation, and sequencing of cDNA's to identify and localize the 5' termini of simian virus 40 early mRNA's. We have examined polyadenylated RNAs obtained from whole cytoplasm and polysomes of two transformed lines and from the cytoplasm of infected cells early and late in the lytic cycle, and we have attempted to correlate the results of our cDNA analyses with recent analyses of early cap structures. We have found that early mRNA's from transformed cells have three principal 5' termini, at residues 5,150, 5,154, and 5,155, with terminal transcribed sequences of CU, GC, and GG, respectively. These termini lie 21 to 26 nucleotides downstream from the early Hogness-Goldberg sequence. Transformed cell early mRNA's also contain a series of less abundant 5' termini that are copied from DNA sequences as far as 80 nucleotides downstream and a minimum of 70 to 75 nucleotides upstream from the Hogness-Goldberg sequence. The templates for the upstream 5' termini and the late simian virus 40 mRNA's overlap by a minimum of 60 to 65 nucleotides. Early mRNA's isolated from cells early in infection contain the same three principal 5' termini and downstream minor 5' termini as transformed cell mRNA's, but they lack 5' termini upstream from the Hogness-Goldberg sequence. With the onset of the late lytic phase, there is a progressive decrease in the utilization of the three principal 5' termini and additional downstream 5' termini and a progressive increase in the utilization of four major termini at residues 5,190 to 5,194, which are 10 to 15 nucleotides upstream from the Hogness-Goldberg sequence. This shift is evident in cells infected with a tsA mutant at the permissive temperature, but is aborted by growth at or shift-up to a restrictive temperature. Thus, this shift is mediated by the gene A product, large T antigen. We present two models, which are mutually exclusive, to account for the role of T antigen in the early-late shift. One involves transcription late in infection on a new DNA template synthesized during DNA replication. The second involves inhibition of initiation of early transcription at residues 5,150 to 5,155 and other downstream sites and a shift of transcription initiation principally to the upstream sites as a result of the binding of T antigen to two sites on simian virus 40 DNA downstream from the Hogness-Goldberg sequence. (Author abstract) (53 Refs)

33. PREFERENTIAL ASSOCIATION OF URACIL-DNA GLYCOSYLASE ACTIVITY WITH REPLICATING SV40 MINICHROMOSOMES.

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FEBS Lett; 133(1):89-91 1981

The preferential association of a major DNA repair enzyme, uracil-DNA glycosylase, with replicating SV40 minichromosomes in investigated. A nuclear extract containing SV40 minichromosomes, but free of cellular DNA and mitochondria was centrifuged to separate replicating and mature minichromosomes. Uracil-DNA glycosylase was tested by collecting fractions from the nuclear extract and incubating with an assay buffer and d3H uracil monophosphate (dUMP) containing DNA. The release of acid or ethanol soluble radioactivity was monitored. Cytochrome c oxidase was determined in aliquots of 100 ul from extracts or gradient fractions. To label both replicating and mature SV40 chromatin, SV40 infected CV-1 cells were incubated with 14C thymidine. Nuclear extracts were prepared as above. Uracil-DNA glycosylase activity was measured in each fraction. Results showed that uracil-DNA glycosylase cosediments with replicating chromatin; a smaller fraction cosedimented with mature chromatin. Thin layer chromatography of the ethanol soluble radioactivity released showed 90% comigrated with uracil. No cytochrome c oxidase activity cosedimented with SV40 minichromosomes. Apparently cosedimentation of uracil-DNA glycosylase with replicating chromatin is not due to contamination by mitochondria. The results suggest that uracil-DNA glycosylase is preferentially associated with replicating SV40 minichromosomes. This may indicate that misincorporation of dUMP is a more frequent event than cytosine deamination. (24 Refs)

34. STIMULATION OF POLYOMA DNA REPLICATION IN ISOLATED NUCLEOPROTEIN COMPLEXES BY FACTORS FROM DROSOPHILA EMBRYOS.

Mastromei G, Eliasson R, Reichard P
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J Mol Biol; 151(4):627-643 1981

Nucleoprotein complexes containing both Form I and replicative intermediates of polyoma DNA prepared from nuclei of virus-infected mouse fibroblasts retain a limited ability to elongate progeny strands of the replicative intermediates. Compared to isolated nuclei, both the rate and the extent of strand elongation is greatly decreased. The isolated complexes synthesize initiator RNA and start new Okazaki fragments, but are deficient in the joining of these fragments. Addition of small amounts of an extract from 16 hr old Drosophila embryos corrects the deficiencies. The stimulatory activity of the extract can be partially purified and has been separated into two fractions by chromatography on Sepharose 6B. With immunological techniques we demonstrate that the mouse DNA polymerase-alpha, tightly bound to the complexes, is responsible for DNA strand elongation. The Drosophila alpha-polymerase present in one of the two fractions purified on Sepharose 6B cannot substitute for the mouse enzyme. The stimulatory activity of the Drosophila fractions is thus not due to alpha-polymerase. (Author abstract) (21 Refs)

35. USE OF WHOLE-CELL FIXATION TO VISUALIZE REPLICATING AND MATURING SIMIAN VIRUS 40: IDENTIFICATION OF NEW VIRAL GENE PRODUCT.

Jackson V, Chalkley R
Dept. Biochemistry, Sch. Medicine, Univ. Iowa, Iowa City, IA, 52242
Proc Natl Acad Sci USA; 78(10):6081-6085 1981

Formaldehyde fixation of simian virus 40 (SV40)-infected CV-1 cells at appropriate times after infection permits us to isolate crosslinked complexes of SV40 minichromosomes during the time of DNA replication and during packaging with viral proteins. Such crosslinked complexes can be separated on the basis of density on CsCl/guanidine.HCl density gradients. During the course of these studies we observed the presence of a low mol wt protein in a region of the gradient much enriched with viral nucleoproteins. This protein is present only in infected cells and has a mol wt and amino acid composition consistent with it being the product of the so-called SV40 agnogene. (Author abstract) (21 Refs)

36. SYNTHESIS OF STABLE UNSPLICED MRNA FROM AN INTRONLESS SIMIAN VIRUS 40-RAT PREPROINSULIN GENE RECOMBINANT.

Gruss P, Efstratiadis A, Karathanasis S, Konig M, Khoury G
Lab. Molecular Virology, NCI, Bethesda, MD, 20205
Proc Natl Acad Sci USA; 78(10):6091-6095 1981

We constructed a recombinant DNA molecule carrying the coding region and the 3' flanking sequences of the rat preproinsulin I gene, replacing the late region of a simian virus 40 (SV40) viral vector. The sequences making up the single intron of the preproinsulin I gene, located within the nontranslated leader region, as well as all of the 5' flanking sequences, have been deleted. The sequences remaining within the late transcriptional unit of SV40 do not retain a functional intron. This 'intronless' mutant nevertheless directs the synthesis of substantial quantities of unspliced stable hybrid transcripts that are translated into polypeptides with insulin immunoreactivity. Two novel 5' termini, not used in wild-type SV40 transcripts, have been detected in the majority of the hybrid RNA molecules. These are located at analogous positions in both of the late SV40 72-base-pair repeats. (Author abstract) (34 Refs)

37. STRUCTURE OF SIMIAN VIRUS 40 (SV40) CHROMOSOME. I. HETEROGENEITY AND COMPOSITION OF NUCLEAR DNA-PROTEIN COMPLEXES AT DIFFERENT STAGES OF LYTIC INFECTION.

Bakaev VV, Nedospasov SA, Georgiev GP
Inst. Molecular Biology, Moscow, USSR
Mol Biol (Mosk); 15(4):939-949 1981

Isolation of virus nucleoproteins from cells infected with simian virus 40 (SV40) showed the presence of five histone fractions. The replicative fractions contained SV40 DNA molecules of various length in complex with histones and nonhistone proteins. At the late stages of lytic infection, approx 90% of nuclear DNA was detected in virions. (26 Refs) (In RUS)

38. SEQUENTIAL TRANSCRIPTION-TRANSLATION OF SIMIAN VIRUS 40 BY USING MAMMALIAN CELL EXTRACTS.

Cepko CL, Hansen U, Handa H, Sharp PA
Center for Cancer Res., Massachusetts Inst. Technology, Cambridge, MA, 02139
Mol Cell Biol; 1(10):919-931 1981

The expression of Simian virus 40 (SV40) was studied using the current technologies of recombinant DNA and in vitro transcription systems. The in vitro transcription of specific SV40 RNAs is described. A polypeptide comigrating with the 94K T antigen of SV40 was not present in products transcribed from Eco-RI-, PstI-, or TaqI-cleaved DNA. However, if a small fraction of the RNA transcribed from the Eco-RI-cleaved DNA had been processed by excision of the intervening sequences between 0.60 an 0.53 monitor units, synthesis of T (94K) would have been possible. Synthesis of the large T family of polypeptides is described. When the relative efficiencies of in vivo- and in vitro-synthetized RNA were quantitated, it was determined that in vitro small tumor antigen t messenger RNA (mRNA) is more efficiently translated than in vivo t mRNA. The translatability of the in vitro transcripts from the SV40 late regions was examined; the synthesis of the viral capsids VP1, VP2, and VP3 from RNA transcribed in vitro was studied. By using DNA templates cleaved within the late region, information concerning the identity of the transcripts that served as VP1, VP2, and VP3 messages was obtained. RNAs synthesized in vitro were fractionated by size to allow the direct identification of VP1, VP2, VP3, and t mRNAs. Other topics discussed include the efficiency of in vitro RNAs as messages, assay for RNA splicing, initiation at internal AUG codons, synthesis of polypeptides by minor transcripts (assay for proteins specified by complementary DNA clones), and lack of termination in the inhibition of translation. (32 Refs)

39. EXPRESSION OF SV40 T ANTIGEN POLYPEPTIDES IN CELLS BIOCHEMICALLY TRANSFORMED BY PLASMIDS CONTAINING THE HERPES SIMPLEX VIRUS THYMIDINE KINASE GENE AND THE GENOME OF AN SV40TSA MUTANT.

Kit S, Otsuka H, Qavi H, Trkula D, Dubbs DR
Div. Biochemical Virology, Baylor Coll. Medicine, Houston, TX, 77030
Int J Cancer; 28(4):485-496 1981

To study the expression of SV40 tsA genomes that had been non-selectively introduced into mouse cells, SV40 tsA207 DNA was cleaved with BamH I and ligated to BamH I-cleaved plasmid pAGO DNA, which contains a functional HSV-I thymidine kinase (TK) gene in the form of a 2 kbp Pvu II fragment inserted at the Pvu II site of pBR322. Recombinant plasmids (11-12 kbp) were isolated and amplified in Escherichia coli K12 strain RRI. Restriction nuclease analyses demonstrated that recombinant plasmids pSB15 and pSB10 contained intact SV40 genomes with the polarity of transcription oriented in the same direction (clockwise) or the opposite direction (counterclockwise), respectively, in relation to that of the HSV-I TK gene. Cla I-cleaved pSB10 and pSB15 DNAs were used to transform LM(TK-) cells to TK+. Serological and disc PAGE analyses showed that clonal lines transformed by these plasmids all expressed the selected marker, SHV-1 TK. Molecular hybridization experiments showed that transformed clonal lines TF pSB10 C7 and TF pSB15 C10 had integrated intact SV40 genomes at one integration site, TF pSB10 C3 had integrated an SV40 genome with a small deletion near the BamH I site, but TF pSB15 C1 had integrated a plasmid from which most of the SV40 nucleotide sequences had been deleted. IF assays with hamster anti-SV40 tumor sera showed that TF pSB10 C7 and TF pSB15 C10 strongly expressed SV40 T antigens in over 90% of the cells, TF pSB10 C3 expressed SV40 T antigens in a minority of the cells, and TF pSB15 C1 did not express SV40 T antigens at all. [35S]-methionine labeling and immunoprecipitation experiments showed that, at 36.5 C: (1) TF pSB10 C7 and TF pSB15 C10 expressed 92K and 20K mol wt species of SV40 T antigens and 50-55K cellular protein; (2) expression of all three was reduced in TF pSB10 C3 cells; and (3) TF pSB15 C1 expressed none of the SV40 T antigens, nor did parental LM(TK-) or TF 8-2 transformed cells (which contained the HSV-1 TK gene but not SV40 DNA). At 40 C, labeling of the 50-55K cellular protein was markedly reduced in TF pSB10 C7 and pSB15 C10 cells. The results suggest that SV40 large T antigen (92K) induces and/or stabilizes the 50-55K cellular protein in these mouse cells. (Author abstract) (53 Refs)

40. PARTIAL PURIFICATION OF SV40 A PROTEIN AND A RELATED CELLULAR PROTEIN FROM PERMISSIVE CELLS.

Tegtmeyer P, Andersen B
Dept. Microbiology, State Univ. New York, Stony Brook, NY, 11794
Virology; 115(1):67-74 1981

A simple and reproducible method for the purification of SV40 A protein (T antigen) from permissive cells has been developed. Two cellular proteins are also purified in smaller quantities by the same procedure. The cellular proteins have mol wt similar to undegraded SV40 A protein. Peptide maps of the viral and cellular proteins are not similar but one of the cellular proteins apparently shares limited antigenicity with the SV40 A protein. (Author abstract) (10 Refs)

41. ALTERNATIVE INTERACTIONS OF THE SV40 A PROTEIN WITH DNA.

Tegtmeyer P, Andersen B, Shaw SB, Wilson VG
Dept. Microbiology, State Univ. New York, Stony Brook, NY, 11794
Virology; 115(1):75-87 1981

We have characterized the DNA binding properties of SV40 A protein (T antigen) from permissive cells. The viral protein protects 110-to 115-, 60-to 65-, 40-to 45-, and 30-to 35-bp fragments of SV40 DNA from an excess of DNase I. The 60-to 65-bp fragment extends some 30-35 bp to either side of the BglI site at the origin of replication. DNase footprinting confirms the location of this binding region and shows that it is the most completely protected DNA binding site under our conditions. The A protein selectively recognizes the 60-to 65-bp origin fragment in the complete absence of adjacent DNA and binds to it in three alternative ways that protect 60-65, 40-45, or 30-35 bp from nuclease. Thus these three interactions with isolated origins recapitulate the binding of viral protein to intact SV40 DNA. We propose that the alternative interactions of SV40 A protein with the 60-to 65-bp origin fragment may represent three different conformational alignments of protein with DNA rather than a simple repetition of a single kind of binding interaction. Viral protein also protect pBR322 and lambda phage DNA from nuclease to a limited extent. Similar interactions could possibly alter the function of DNA control regions of host cells. (Author abstract) (15 Refs)

42. BINDING OF DEPHOSPHORYLATED A PROTEIN TO SV40 DNA.

Shaw SB, Tegtmeyer P
(c/o Tegtmeyer) Dept. Microbiology, State Univ. New York, Stony Brook, NY, 11794
Virology; 115(1):88-96 1981

We have examined the role of SV40 A protein (T antigen) phosphorylation in the specific binding of the protein to SV40 DNA using protein that was dephosphorylated in vitro. Alkaline phosphatase removed more than 75% of the phosphate radiolabel associated with the A protein. Most of the phosphoserine residues were sensitive to the enzyme while most of the phosphothreonine residues were resistant. Phosphopeptide mapping of the dephosphorylated protein demonstrated that the phosphate groups at individual sites were either highly sensitive or highly resistant to the phosphatase. Thus, enzymatically dephosphorylated A protein provides a useful probe for analysis of the function of specific phosphorylation sites. Dephosphorylated A protein, like untreated A protein, preferentially bound a restriction fragment of SV40 DNA that contains the origin of DNA replication and sequences involved in transcriptional control. The binding was characterized further using a DNase protection assay and DNase footprinting. Dephosphorylated A protein protected SV40 DNA in a manner that was indistinguishable from that of untreated protein. The effects of A protein concentration and salt treatment on binding were the same for phosphatase-treated and control untreated A protein under the conditions of our assay. These results suggest that the location, alignment, and affinity of binding are unaffected by dephosphorylation. We conclude that phosphorylation at the majority of the phosphoserine sites is not directly involved in the regulation of site-specific recognition and binding of the A protein to SV40 DNA. (Author abstract) (23 Refs)

43. REPLICATION OF POLYOMA DNA IN NUCLEI ISOLATED FROM AZIDOCYTIDINE-INHIBITED FIBROBLASTS.

Eliasson R, Pontis E, Reichard P, Eckstein F
Dept. Biochemistry, Medical Nobel Inst. Karolinska Inst., S-104 01 Stockholm, Sweden
J Biol Chem; 256(17):9044-9050 1981

The compound 2'-deoxy-2'-azidocytidine was earlier shown to inhibit the synthesis of DNA (but not RNA or protein) in polyoma virus-infected 3T6 fibroblasts. The diphosphate of azidocytidine is a known inhibitor of ribonucleotide reductase, but deoxyribonucleotide pools of inhibited cells were only marginally affected. Nuclei isolated from the inhibited cells incorporated dNTPs into polyoma DNA at a fairly normal rate with incorporation occurring mainly around the origin of replication. This led to the earlier suggestion that azidocytidine interferes with origin initiation. We now investigate polyoma DNA replication in nuclei isolated after azidocytidine inhibition of cells from a hydroxyurea-resistant cell line that contains an increased activity of ribonucleotide reductase. The nuclei rapidly incorporated labeled dNTPs into polyoma DNA progeny strands which between 10 sec and 15 min grew from an av length of 100 to 1,500 nucleotides. Incorporation initially occurred to a large extent around the origin of replication, but a substantial part of isotope was also recovered more distant from the origin. All strands were elongated at similar rates irrespective of their location. With 5-bromodeoxyuridine triphosphate replacing dTTP, only 28% of the thymine of progeny strands was substituted by bromouracil after 20 sec of incubation. This value increased to 76% after 15 min. These data demonstrate that synthesis in isolated nuclei starts from short primers, preferentially but not exclusively located in the origin region, and suggest that azidocytidine in intact cells inhibits chain elongation and not origin initiation. (Author abstract) (26 Refs)

44. COMPARISON OF PHOSPHORYLATION OF TWO POLYOMA VIRUS MIDDLE T ANTIGENS IN VIVO AND IN VITRO.

Schaffhausen B, Benjamin TL
Dept. Pathology, Harvard Medical Sch., Boston, MA, 02115
J Virol; 40(1):184-196 1981

Two species of polyoma virus middle T antigen were detected in both lytically infected and transformed cells by in vitro kinase assay of immunoprecipitates. A minor species with an apparent mol wt of 58,000 (58K) represented less than 10% of the total middle T protein. This species was roughly 10x more active as a phosphate acceptor than was the predominant 56K form. Partial proteolytic mapping experiments showed that the same site was phosphorylated in both species. Mapping of the middle T antigens from a series of deletion mutants suggested

that the major site of phosphorylation is tyrosine residue 315. Phosphorylation occurred on both middle T species in vivo, involving sites predominantly other than the tyrosine labeled in vitro. The 56K and 58K middle T forms differed from each other in their in vivo phosphorylation patterns. Some phosphate was incorporated into the 58K species in a region of the molecule to which at least part of the apparent mol wt difference could be mapped. hr-t mutant NG-59, which codes for a slightly altered middle T, produced only a single species (56K) which was inactive in the in vitro kinase reaction. Moreover, no 58K species appeared in vivo with this mutant.The hr-t mutants are therefore defective in both aspects of phosphorylation. Phenotypically normal revertant cells of a polyoma transformed line failed to express any middle T antigens or associated kinase activity. (Author abstract) (43 Refs)

45. DNA SYNTHESIS AND TEMPLATE ACTIVITY IN A MUTANT OF POLYOMA VIRUS WITH ALTERED EXPRESSION OF LATE VIRAL PROTEINS.

Blackburn C, Hare JD
Dept. Microbiology, Univ. Rochester Medical Center, Rochester, NY, 14642
J Virol; 40(1):142-149 1981

The 3049 strain of polyoma virus overproduces late RNA and proteins. The synthesis and accumulation of virus DNA was measured in cells infected with 3049 and a wild-type virus, lpS, to assess the possible role of gene dosage in this phenomenon. The rate of incorporation of [3H]thymidine into the DNA of 3049 and lpS, to assess the possible role of gene dosage in this phenomenon. The rate of incorporation of [3H]thymidine into the DNA of 3049 and lpS virus was shown to be identical. The number of genome copies per cell, determined by DNA-DNA reassociation kinetics, was found to be similar in either whole cell or Hirt extracts of cells infected with the two viruses. Viral transcription complexes were isolated by Sarkosyl extraction, and the specific transcriptional activity was measured by incorporation of [3H]UTP. The incorporation of [3H]UTP and the DNA content of transcription complexes were indistinguishable. These results suggest that overproduction of late RNA (and proteins) by the 3049 virus is not due to either an increased number of viral genomes per cell or an increased fraction of genomes involved in transcription. These and other data (unpublished) support the conclusion that the altered phenotype is due to a posttranscriptional mechanism. (Author abstract) (29 Refs)

46. EXPRESSION OF EARLY ADENOVIRUS GENES REQUIRES A VIRAL ENCODED ACIDIC POLYPEPTIDE.

Ricciardi RP, Jones RL, Cepko CL, Sharp PA, Roberts BE
Wistar Inst. Anatomy and Biology, 36th St. at Sprace, Philadelphia, PA, 19104
Proc Natl Acad Sci USA; 78(10):6121-6125 1981

Host-range mutants of adenovirus 5 that contain a defect in region E1A (0-4.5 units) fail to replicate in HeLa cells and to transform rodent cells. In HeLa cells, these mutants synthesize only the two RNAs from E1A that share the same 5' and 3' termini but differ in length by the amount of internal sequence removed by splicing. RNA from wild-type virus, selected by hybridization to DNA from region E1A, translates into polypeptides of Mr 51,000 and 48,000 that are highly acidic in isoelectric focusing gels. These acidic Mr 51,000 and Mr 48,000 polypeptides are encoded by the longer and shorter E1A RNAs, respectively. Two of the host-range mutants, H5hr1 and H5hr2, fail to synthesize the Mr 51,000 polypeptide but do produce the Mr 48,000 polypeptide and a novel polypeptide thought to be a truncated portion of the Mr 51,000 polypeptide. H5hr1 and H5hr2 are hypothesized to have termination codons in sequences found only in RNA encoding the Mr 51,000 polypeptide. This prediction is verified for H5hr1 by DNA sequence analysis. The other three host-range mutants (H5hr3-5) synthesize both acidic polypeptides and are predicted to be missense. These results strongly imply that the Mr 51,000 polypeptide, alone or in combination with the Mr 48,000 polypeptide, is needed to regulate expression of adjacent viral genes during the early phase of adenovirus infection. (Author abstract) (34 Refs)

47. HERPES SIMPLEX VIRUS TYPES 1 AND 2 COMPLETELY HELP ADENOVIRUS-ASSOCIATED VIRUS REPLICATION.

Buller RM, Janik JE, Sebring ED, Rose JA
Lab. Biology Viruses, Natl. Inst. Allergy and Infectious Diseases, Bethesda, MD, 20205
J Virol; 40(1):241-247 1981

In addition to adenoviruses, which are capable of completely helping adenovirus-associated virus (AAV) multiplication, only herpesviruses are known to provide any AAV helper activity, but this activity has been thought to be partial (ie, AAV DNA, RNA, and protein syntheses are induced, but infectious particles are not assembled). In this study, however, we show that herpes simplex virus Type 1 (HSV-1) and Type 2 (HSV-2) are in fact complete AAV helpers and that AAV Type 2 (AAV2) infectivity yields can approach those obtained when coinfections are carried out with a helper adenovirus. AAV helper activity was demonstrated in KB cells with two HSV-1 strains (11124 and 17MP) and an HSV-2 strain (HG52). Each herpesvirus supported AAV2 multiplication with comparable efficiency. AAV2 multiplication was similarly efficient in HSV-1 coinfections of HeLa cells, whereas lower yields were obtained in HEp-2 and primary human embryonic kidney cells. HSV-1 also supported AAV-1 multiplication in HeLa cells but, at corresponding multiplicities of infec tion, AAV1 grew less efficiently than AAV2. Comparisons of the time courses of AAV2 DNA, RNA, and protein syntheses after coinfection with either adenovirus Type 5 or HSV-1 revealed that, in each case, the onset of synthesis and attainment of max synthesis rate occurred earlier in coinfections with HSV-1. These findings demonstrate the linkage of AAV macromolecular synthesis to an event(s) in the helper virus cycle. Aside from this temporal association, helper-related differences in AAV macromolecular synthesis were not apparent. (Author abstract) (22 Refs)

48. DEGRADATION OF INTRACELLULAR DNA IN KB CELLS INFECTED WITH CYT MUTANTS OF HUMAN ADENOVIRUS TYPE 12.

Ezoe H, Lai Fatt RB, Mak S
Inst. Res. and Development, Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan
J Virol; 40(1):20-27 1981

A group of mutants (cyt mutants) with much reduced oncogenicity was isolated from the highly oncogenic human adenovirus Type 12. These mutants induce extensive cellular destruction during lytic infection of human cells and produce low yields of virions. We report here that human KB cells infected with cyt mutants synthesized a reduced amount of viral DNA as compared with cells infected with the parental virus. Furthermore, the newly synthesized viral and cellular DNAs were extensively degraded in mutant-infected cells. Viral DNA was first synthesized as complete genome size, and most of it was

degraded to subgenomic size within 6 hr after synthesis. This virus-induced DNA degradation function, as well as the low yield of virions, was prevented by co-infection with the parental virus. (Author abstract) (19 Refs)

IMMUNOLOGICAL STUDIES

49. HERPESVIRUS ATELES AND HERPESVIRUS SAIMIRI TRANSFORM MARMOSET T CELLS INTO CONTINUOUSLY PROLIFERATING CELL LINES THAT CAN MEDIATE NATURAL KILLER CELL-LIKE CYTOTOXICITY.

Johnson DR, Jondal M
Dept. Pathology and Lab. Medicine, Univ. Nebraska Medical Center, 42nd and Dewey Ave., Omaha, NE, 68105
Proc Natl Acad Sci USA; 78(10):6391-6395 1981

Herpesvirus ateles (HVA) and herpesvirus saimiri (HVS) have the capacity to transform cotton-topped marmoset T lymphocytes into continuously proliferating cell lines that retain some functions associated with cell-mediated immunity. In the present paper, we demonstrate that HVA/HVS-transformed T cell lines are cytotoxic in a short-term 51Cr release assay and that this killing resembles killing by marmoset natural killer (NK) cells. The relationship between NK cells and HVA/HVS-transformed killer cell lines is discussed in view of present knowledge of the origin and function of the NK system. It is suggested that the described cytotoxic cell lines may be useful for further defining cellular cytotoxicity with regard to cell surface recognition, regulatory events, and lytic mechanisms. (Author abstract) (37 Refs)

50. MONOCLONAL ANTIBODY AGAINST A BURKITT LYMPHOMA-ASSOCIATED ANTIGEN.

Wiels J, Fellous M, Tursz T
Laboratoire d'Immunologie Clinique, Institut Gustave-Roussy, 94800 Villejuif, France
Proc Natl Acad Sci USA; 78(10):6485-6488 1981

A monoclonal antibody, referred to as 38.13, was obtained by fusing murine myeloma cells with Lewis rat splenocytes sensitized with Daudi cells (human Burkitt lymphoma containing Epstein-Barr virus genome but lacking HLA-A, -B, and -C and beta2-microglobulin molecules at the cell surface). 38.13 antibody was demonstrated to be a rat IgM. By complement-dependent microcytotoxicity and indirect immunofluorescence assays, 38.13 antibody was shown to react specifically with cells derived from Burkitt tumors, including both Epstein-Barr virus genome-carrying and Epstein-Barr virus-negative Burkitt lymphoma. By contrast, Epstein-Barr virus-containing lymphoblastoid cell lines derived from normal B lymphocytes were not recognized by 38.13 antibody. Fresh malignant cells from patients affected with various lymphoproliferative disorders were negative, except 4/8 having abdominal Burkitt-like lymphomas. Normal lymphocytes from peripheral blood, spleen, lymph node, tonsil, and bone marrow and mitogen (phytohemagglutinin, pokeweed mitogen, and concanavalin A)-activated blasts were also negative. Thus, 38.13 antibody apparently recognized a Burkitt-associated antigen that is not related to Epstein-Barr virus. The pattern of reactivity of 38.13 antibody with various Burkitt lymphoma cells appeared quite heterogenous and some Burkitt cells were consistently negative. 38.13 antibody thus defines a subset of Burkitt lymphomas. (Author abstract) (18 Refs)

OTHER RELATED STUDIES

51. CLONED MOUSE MAMMARY TUMOR VIRUS DNA IS BIOLOGICALLY ACTIVE IN TRANSFECTED MOUSE CELLS AND ITS EXPRESSION IS STIMULATED GLUCOCORTICOID HORMONES (MEETING ABSTRACT).

Buetti E, Diggelmann H
Institut Suisse de Recherches Experimentales sur le Cancer, CH-1066 Epalinges, Switzerland
Experientia; 37(6):640 1981

We have cloned circular unintegrated mouse mammary tumor virus (MMTV) DNA from infected rat hepatoma cells in bacteriophage lambda. Seven independent clones containing MMTV DNA of homogeneous length of 9 kilobase (kb) (5) or 10 kb (2) were identified. The 5 9-kb clones had identical restriction maps consistent with that of 9 kb unintegrated DNA, the other 2 were aberrant. MMTV DNA inserts were purified, ligated and used for cotransfection of Ltk- cells together with a plasmid containing the thymidine kinase gene of herpes simplex virus. All Tk+ cell clones acquired new MMTV sequences and those transfected with the 9 kb MMTV DNA synthesized normal viral RNA and proteins. Viral gene expression was increased by the addition of dexamethasome. (no Refs)

52. THE CARCINOGEN N-ACETOXY-ACETYLAMINOFLUORENE REACTS PREFERENTIALLY WITH A CONTROL REGION OF THE INTRACELLULAR SIMIAN VIRUS 40 CHROMOSOME (MEETING ABSTRACT).

Beard P, Kaneko M, Cerutti P
No affiliation given
Experientia; 37(6):638 1981

We have investigated the binding of the ultimate carcinogen N-acetoxy-acetylaminofluorene (AAAF) to specific regions of the SV40 chromosome in situ in the intact infected cell. SV40-infected cells late in the lytic cycle were incubated with 3H-AAAF. SV40 DNA was extracted, digested with restriction enzymes Hae III and Kpn I and radioactivity in each DNA fragment determined. The results indicated that a stretch of DNA near the origin of replication of the intracellular SV40 chromosome was more susceptible to attack by AAAF than the rest of the SV40 genome. When naked DNA was labeled with 3H-AAAF in vitro no hyperreactive region was seen. The hyperreactive region may represent a stretch of DNA which is nucleosome-free or has another unusual chromosomal structure. (no Refs)

53. HOWLER MONKEY-MOUSE T CELL HYBRIDOMAS CARRYING HERPESVIRUS SAIMIRI (HVS) GENOME (MEETING ABSTRACT).

Rangan SR, Martin LN, Gormus BJ
Tulane University, Covington, LA, 70433
In Vitro; 17(3):228 1981

In an attempt to study latency of a primate T lymphotropic herpesvirus, monkey-mouse T cell hybridoma lines have been developed. Cryopreserved HVS genome-carrying splenic lymphocytes (3 x 10(7)) from a howler monkey (Alouatta caraya) with virus-induced lymphoma were fused with 3 x 10(6) BW5147 (TGr) mouse lymphoma cells in the presence of polyethylene glycol 1000. Hybrids were grown as microcultures in

a selective medium containing hypoxanthine, aminopterin and thymidine. Cell growth occurred in 38 of 732 wells initiated. While 15 of 35 hybridoma lines tested by rosette formation with aminoethylisothiouronium bromide-treated sheep RBC contained from 10 to 47% rosette-forming cells characteristic of primate T cells, others were negative for such cells. HVS was recovered from 10 of 36 hybridomas tested by co-cultivation with susceptible primate cells. In the virus positive lines, only a small proportion of the cells induced HVS-related cytopathic effect in co-cultures and expressed viral antigens by immunofluorescence. (no Refs)

54. STUDIES TO ELUCIDATE SUSCEPTIBILITY DETERMINANTS FOR MOUSE EPIDERMAL CARCINOGENESIS (MEETING ABSTRACT).

Strickland JE
National Cancer Institute, Bethesda, MD, 20205
Symposium on Host Factors in Human Carcinogenesis, June 8-11, 1981, Cape Sunion, Greece International Agency for Research on Cancer; 1981

Recent studies in our laboratory demonstrated that SENCAR mice are markedly susceptible to two-stage skin carcinogenesis when compared to BALB/c mice. Sensitivity appears to be determined by the target tissue since SENCAR skin grafted to nude mice developed papillomas at a high frequency after initiation and promotion whereas BALB/c skin grafts developed no tumors. DNA repair capacity was studied in SENCAR and BALB/c epidermal cells in culture. Host cell reactivation, utilizing UV light-irradiated herpes simplex virus, was similar in cells of both strains. SENCAR cells have a 1.2-2.0 X greater binding capacity for epidermal growth factor than BALB/c cells. However, the increased binding in response to retinoic acid and the rapid decrease after phorbol ester exposure are similar in the two strains. Epidermal cells of both strains contain proviral DNA sequences for xenotropic type C RNA viruses, but spontaneous expression occurs more readily in BALB/c. AKR and Rauscher murine leukemia viruses infect and replicate well in epidermal cell cultures of both BALB/c and SENCAR. The frequency of spontaneous and carcinogen-induced, differentiation-resistant foci in vitro is greater in SENCAR than in BALB/c epidermal cells. These results suggest that susceptibility for skin carcinogenesis in SENCAR mice is determined by the target tissue itself and has no clear relation to DNA excision repair, endogenous virus complement, or epidermal growth factor receptors. (3 Refs)

55. INSERTION OF NEW GENES INTO BONE MARROW CELLS OF MICE.

Cline MJ, Stang HD, Mercola K, Salser W
Dept. Medicine, Univ. California, Center for Health Sciences, Los Angeles, CA, 90024
Haematol Blood Transfus; 26:167-170 1981

Utilizing existing techniques for inserting new genes into bone marrow stem cells of living mice and for favoring the proliferation of transformed stem cells carrying the new genes, experiments were carried out to insert the mouse dihydrofolate reductase (DHFR) genes resistant to methotrexate (MTX) and the herpes simplex virus thymidine kinase (HSV-tk) genes into mouse stem cells. The insertion of the MTX-resistant DHFR genes permitted the use of MTX challenge to favor the proliferation of transformed cells. The simultaneous insertion of HSV-tk genes permits the transformed cells to grow in the presence of MTX by utilizing the salvage pathway of DNA synthesis more efficiently. Karyotype analysis confirmed the existence of transformed stem cells. Efficient gene expression was obtained for both of the inserted gene sequences. This technique may have direct clinical application in treating hemoglobinopathies and hematopoietic malignancies and in reducing the hematopoietic toxicity of anticancer drugs. (11 Refs)

56. VIRUS AND RECOMBINANT DNA.

Zhu J
Inst. Virology, Chinese Acad. Medical Sciences, Beijing, People's Republic of China
Zhonghua Weishengwuxue He Mianyixue Zazh; 1(1):1-6 1981

Research and recent advances in recombinant DNA technics are reviewed. Major topics covered include: (1) use of recombinant DNA in cloning viral genes, notably hepatitis B virus are influenza viruses; and (2) use of recombinant DNA from animal viruses in the study of gene expression and cell transformation as with SV40 viral products and the thymidine kinase gene of herpesviruses. (20 Refs) (In CHI)

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DNA TUMOR VIRUSES IN NON-PRIMATE SYSTEMS

SERIES CB11 No. 62/04 APRIL, 1982

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VIRUS ISOLATION/ STRUCTURE/ CHARACTERIZATION/ IDENTIFICATION

1. SITE-SPECIFIC INVERSION SEQUENCE OF THE HERPES SIMPLEX VIRUS GENOME: DOMAIN AND STRUCTURAL FEATURES.

Mocarski ES, Roizman B
Marjorie B. Kovler Viral Oncology Lab., Univ. Chicago, 910 East 58th St., Chicago, IL, 60637
Proc Natl Acad Sci USA; 78(11,part2):7047-7051 1981

The genome of herpes simples virus-1 consists of two covalently linked components, L and S, that invert relative to each other. The L and S components consist of unique DNA sequences bracketed by inverted repeats. The inverted repeats of the L component are designated ab and b'a' and those of the S component are designated a'c' and ca. The number of a sequences at the termini and at the L-S component junction varies from one to several copies. Insertion into the middle of the L component of a DN A fragment consisting of 156 base pairs (bp) of the b sequence, an entire a sequence of 501 bp, and 618 bp of the c sequence created a new site through which additional inversions in the genome occurred. Comparison of

HE 20.3123/2:

the nucleotide sequences of DNA fragments containing one and two a sequences defined the domain of the a sequence. The single a sequence consists of two 20-bp direct repeats (designated as DR1) bracketing a region that contains 19 tandem direct repeats of a 12-bp sequence (DR2) adjacent to three direct repeats of a 37-bp sequence (DR4), in addition to short stretches of unique sequences. The fragment with two tandem a sequences contained three copies of DR1-ie, the intervening DR1 was shared by the two a sequences. Furthermore, one a sequence contained 22 copies of DR2 and two copies of DR4 whereas the second a sequence contained 19 copies of DR2 and two copies of DR4. These observations suggest that (i) amplification of the number of terminal and internal a sequences is the consequence of intramolecular or intermolecular recombination through DR1, (ii) the number of copies of DR2 and DR4 within the a sequence is not fixed and may vary as a consequence of unequal crossing over or slippage, and (iii) inversion results from intramolecular recombination between terminal and inverted a sequences. (Author abstract) (16 Refs)

2. NUCLEOTIDE SEQUENCES OF THE JOINT BETWEEN THE L AND S SEGMENTS OF HERPES SIMPLEX VIRUS TYPES 1 AND 2.

Davison AJ, Wilkie NM
Medical Res. Council Virology Unit, Univ. Glasgow, Church St., Glasgow G11 5JR, England
J Gen Virol; 55(part2):315-331 1981

The a sequence of herpes simplex virus (HSV) is present as a direct repeat at the genomic termini and also in inverted orientation at the joint between the L and S segments. DNA sequences have been determined for the joint regions of the genomes of HSV-1 and HSV-2, and relative to these sequences the genomic termini are in both cases located close to a short direct repeat of 17 to 21 base pairs (bp) at the b-a and a-c junctions. The HSV-1 joint region contains three separate tandem direct reiterations of short sequences, (12, 16 and 17 bp in strain 17) and we conclude that size heterogeneity in the a and c sequences is due to variable copy numbers of these repeated units. It is likely that a considerable part of the HSV-1 joint region does not code for polypeptide. (Author abstract) (54 Refs)

3. HERPES SIMPLEX VIRUS TYPE 1 AND 2 INTRACELLULAR P40: TYPE-SPECIFIC AND CROSS-REACTIVE ANTIGENIC DETERMINANTS ON PEPTIDES GENERATED BY PARTIAL PROTEOLYSIS.

Heilman CJ, Zweig M, Hampar B
Lab. Molecular Virology, Carcinogenesis Intramural Program, NCI, Frederick, MD, 21701
J Virol; 40(2):508-515 1981

Intracellular p40 is a class of protein ranging in mol wt from 39,000 to 45,000 that is immunoprecipitated from herpes simplex virus type 1 (HSV-1)- and HSV-2-infected cell extracts by mouse monoclonal antibodies or guinea pig antisera against HSV-1 and HSV-2 nucleocapsid p40. Analysis by a two-dimensional gel system showed that HSV-1 and HSV-2 intracellular p40 each consisted of three major components. However, these HSV-1 and HSV-2 proteins differed in charge and size. Analysis of Staphylococcus aureus V8 protease partial digests by two-dimensional gel electrophoresis indicated that none of the peptides of HSV-1 and HSV-2 intracellular p40 were identical. Immunoprecipitation of the partial digest products of intracellular p40-1 and p40-2 with homologous and heterologous guinea pig antisera resulted in the precipitation of various combinations of peptides indicating the presence of either type-specific or cross-reactive antigenic determinants. (Author abstract) (17 Refs)

4. ANTIGENIC AND STRUCTURAL CONSERVATION OF HERPESVIRUS DNA-BINDING PROTEINS.

Littler E, Yeo J, Killington RA, Purifoy DJ, Powell KL
Dept. Microbiology, Univ. Leeds, Leeds LS2 9JT, England
J Gen Virol; 56(part2):409-419 1981

Previously, we have shown a common antigen of several herpesviruses (pseudorabies virus, equine abortion virus and bovine mammillitis virus) to be antigenically related to the major DNA-binding proteins of herpes simplex virus Types 1 and 2. In this study we have purified the cross-reacting polypeptide from cells infected with pseudorabies virus, equine abortion virus and bovine mammillitis virus and shown the cross-reacting protein to be a major DNA-binding protein for each virus. Tryptic peptide analysis of the cross-reacting DNA-binding proteins of all five viruses has shown structural similarities. The proteins thus were shown to share common antigenic sites, to have similar biological properties and to have a highly conserved amino acid sequence. This unexpected similarity between proteins from diverse herpesviruses suggests an essential and fundamental role of the major DNA-binding protein in herpes virus replication. (Author abstract) (28 Refs)

5. ANATOMY OF ONCOGENIC TUPAIA HERPESVIRUSES ISOLATED FROM A MALIGNANT LYMPHOMA AND A HODGKIN'S SARCOMA (MEETING ABSTRACT).

Darai G, Matz B, Flugel RM, Delius H
No affiliation given
Tenth International Symposium for Comparative Research on Leukemia and Related Diseases, August 31-September 4, 1981, Los Angeles, California. The Association 187 pp., 1981.

Tupaia (the tree shrew), a member of the family Tupaiidae, is regarded as one of the prosimians that most bridges the gap between insectivores and primates. The isolation and characterization of Tupaia Herpesviruses has been reported. Tupaia Herpesviruses (THV) were efficiently propagated, plaque-purified and cloned on tupaia embryonic fibroblasts. The characterization of the DNA of these Tupaia He rpesviruses was studied. The DNA of THV-2, which was isolated from malignant lymphoma and THV-3, which was isolated from a Hodgkin's sarcoma of tupaia were analyzed in detail. The viral DNAs were cleaved with different restriction endonucleases, and the cleavage sites of Hind III and Eco RI enzymes on THV-2 and 3 DNA were determined. The mol wt of THV DNAs was determined by electron microscopic measurement of the contour length of the DNA, which resulted in 133x10(6) for THV-2 and 130x10(6) for THV-3. Single-stranded DNA of THV was prepared for electron-microscopy by heating the viral DNA in formamide and isolating the single-strands from agarose gel. It was found that single-stranded THV DNA does not display any extended stem-loop structures. With respect to the structure of THV genomes, the Tupaia Herpesviruses are unique when compared to the DNAs of other known herpesviruses from different species. This conclusion was verified by electron microscopic examination of single-stranded THV-DNA under conditions favoring intra-strand hybridization. Self-annealed single strands of THV-DNA did not form stem-loop structures. This result rules out the existence of isomeric forms of THV DNAs. (3 Refs)

6. CLONED FRAGMENTS OF HUMAN ADENOVIRUS TYPE-12 DNA.

Vogel S, Brotz M, Kruczek I, Neumann R, Eick D, Winterhoff U, Doerfler W
Inst. Genetics, Univ. Cologne, D-5000 Cologne 4, W. Germany
Gene; 15(2/3):273-278 1981

The cloning of EcoRI, BamHI, HindIII, and PstI restriction fragments of adenovirus type 12 (Ad12) DNA is described. EcoRI fragment A was found to contain the right terminal 5 kilobase pairs of Ad12 DNA including the terminal 143 base pairs. (26 Refs)

7. GENOMIC ARRANGEMENT OF AN ADENOVIRUS-SIMIAN VIRUS 40 HYBRID VIRUS, AD2+ND4DEL.

Westphal H
Lab. Molcular Genetics, Nat. Inst. Child Health and Human Development, Bethesda, MD, 20205
J Virol; 40(2):526-532 1981

Ad2+ND4del is an adenovirus Type 2-simian virus 40 hybrid virus nondefective for growth in human cells. The virus was first observed when stocks of Ad2+ND4, a hybrid isolated from primary monkey kidney cells, were propagated in human cells. This paper describes the DNA sequence at two sites of DNA recombination, the site of the left adenovirus Type 2-simian virus 40 junction and the site of a deletion of internal simian virus 40 sequences. Since the deletion was observed when the virus was switched from monkey to human cells, an analysis of gene expression in the region of DNA rearrangement may prove useful for the elucidation of molecular events that accompany virus growth in different hosts. (Author abstract) (34 Refs)

VIRUS ASSOCIATED CELL TRANSFORMATION AND HOST ALTERATION

8. FATE OF VIRAL DNA IN NONPERMISSIVE CELLS INFECTED WITH SIMIAN VIRUS 40.

Chia W, Rigby PW
(c/o Rigby) Cancer Res. Campaign, Dept. Biochemistry, Imperial Coll. Science and Technology, London SW7 2AZ England
Proc Natl Acad Sci USA; 78(11,part2):6638-6642 1981

Mouse cells are nonpermissive for simian virus 40 (SV40): replication of viral DNA is undetectable and progeny virions are not produced. Infection leads instead to the establishment of stably transformed cell lines in which viral DNA is covalently integrated into cellular DNA. We have followed the fate of SV40 DNA in infected mouse cells to define steps in viral DNA metabolism that precede integration. A novel high mol wt form of SV40 DNA is synthesized shortly after infection by a process sensitive to the inhibition of DNA replication. This DNA represents polymers in which viral genomes are organized as tandem 'head-to-tail' arrays. Recombination can be demonstrated with mutant viruses, but the recombination frequency is not high enough to account for the synthesis of polymers by recombination between infecting genomes. We conclude that polymers are synthesized by DNA replication and that they then recombine with one another. We believe that the polymers also recombine with cellular DNA and are thus the precursor to integrated viral DNA. Such a model accounts directly for the high frequency of tandemly duplicated viral insertions in transformed cells and also leads to experimentally testable predictions. (Author abstract) (37 Refs)

9. QUANTITATION AND CHARACTERIZATION OF A SPECIES-SPECIFIC AND EMBRYO STAGE-DEPENDENT 55-KILODALTON PHOSPHOPROTEIN ALSO PRESENT IN CELLS TRANSFORMED BY SIMIAN VIRUS 40.

Chandrasekaran K, McFarland VW, Simmons DT, Dziadek M, Gurney EG, Mora PT
(c/o Mora) Macromolecular Biology Section, Div. Cancer Biology and Diagnosis, NCI, NIH, Bethesda, MD, 20205
Proc Natl Acad Sci USA; 78(11,part2):6953-6957 1981

A 55-kilodalton (kDal) protein was detected recently in primary cultures of day 12 mouse embryos by immunoprecipitation with serum from simian virus 40 (SV40) tumor-bearing hamsters (T serum). Preliminary evidence suggested that this protein was similar to a cellular 55-kDal protein induced after SV40 transformation of mouse cells. We now show that specific approx 55-kDal [35S]methionine-labeled proteins precipitate from primary cultures of midgestation mouse, rat, and hamster embryos on addition of T serum or monoclonal antiserum prepared against the SV40-induced mouse 55-kDal proteins. The two-dimensional maps of the [35S]methionine-labeled tryptic peptides of the mouse, hamster, and rat embryo proteins are similar to the maps of the corresponding proteins from SV40-transformed cells. Primary cells from midgestation mouse, hamster, or rat embryos contain one-third to one-half as much 55-kDal protein as a SV40-transformed mouse fibroblast cells and nearly the same amount as F9 mouse embryonal carcinoma cells. The amount of 55-kDal protein is greatly reduced on replating the mouse, rat, or hamster embryo primar y cells. The amount of this protein in mouse embryos is dependent on the stage of the embryo. The embryo proteins are phosphoproteins. (Author abstract) (32 Refs)

10. COMPARISON OF T ANTIGEN-ASSOCIATED HOST PHOSPHOPROTEINS FROM SV40-INFECTED AND -TRANSFORMED CELLS OF DIFFERENT SPECIES.

Fanning E, Burger C, Gurney EG
Fakultat fur Biologie, Universitat Konstanz, D-7750 Konstanz, W. Germany
J Gen Virol; 55(part2):367-378 1981

Simian virus 40 (SV40)-infected and -transformed cells contain, in addition to the virus-coded tumor antigens, one or more 48K to 56K host tumor antigens. At least part of this class of host proteins exists as a fast-sedimenting complex with the SV40 large T antigen. The host proteins associated with the large T antigen in SV40-transformed monkey, mouse and human cells and SV40-infected monkey cells were compared by two-dimensional gel electrophoresis and V8 partial proteolysis peptide mapping. Although these proteins differed slightly in apparent mol wt and peptide pattern, they migrated identically in isoelectric focusing gels. These results suggest that the cellular proteins associated with large T antigen in different hosts are very closely related to each other. Despite their similarities, the 55K proteins from different host cells form complexes of different stabilities with large T antigen, as judged by spontaneous dissociation of the complexes during storage, and the fractions of the 55K cellular protein and large T antigen found free and in the complexed form in each different host cell. (Author abstract) (36 Refs)

11. REPLICATION OF VIRAL DNA SEQUENCES INTEGRATED WITHIN THE CHROMATIN OF SV40-TRANSFORMED CHINESE HAMSTER LUNG CELLS.

Marchionni MA, Roufa DJ
Div. Biology, Kansas State Univ., Manhattan, KS, 66506
Cell; 26(2,part2):245-258 1981

To determine when during S phase integrated viral DNA sequences in several tsA SV40-transformed Chinese hamster cell clones replicate, we pulse-labeled cultures with BrdUrd and subsequently collected mitotic cells during sequential time intervals. Restriction endonuclease mapping indicated that each of the three SV40-transformed Chinese hamster lung cell clones contained a single viral DNA sequence at a different, but in each case unique, chromosomal site. DNA was extracted from each population of mitotic cells and then was resolved into dense, BrdUrd-containing and light, unsubstituted DNA fractions by cesium chloride gradient centrifugation. In each pair of samples obtained, we measured viral DNA sequences by soln hybridization using single-stranded SV40 32P-labeled DNA probes. Our results support the conclusions that specific genes within a mammalian DNA are programmed to replicate at particular times durir.g S phase, and that the SV40 A gene product, large T antigen, programs integrated viral DNA sequences to replicate v ery early in S phase. The fractions of viral DNA replicated early in S phase appeared to correlate with each clone's content of functional large T antigen at permissive and nonpermissive culture temperatures. (Author abstract) (96 Refs)

12. PATTERNS OF CELL COMMUNICATION AND DIFFERENTIATION IN SV40 TRANSFORMED HUMAN KERATINOCYTES.

Steinberg M, Defendi V
Dept. Pathology, New York Univ. Medical Center, New York, NY, 10016
J Cell Physiol; 109(1):153-159 1981

The effect of viral transformation on intercellular communication (IC) and cellular differentiation in human keratinocytes was studied. Human keratinocytes from neonatal foreskin were grown on lethally irradiated 3T3 feeder cell layers. Uninfected cultures were compared with cultures infected with the oncogenic virus SV40. IC was studied by microinjection of fluorescein. Cell maturation was determined by cytochemical staining. Uninfected keratinocytes showed extensive coupling, as indicated by widespread exchange of fluorescein. No communication with mouse feeder cells or human fibroblasts was noted. The effects of SV40 infection appeared after three to four serial passages, when 50-70% of the cells were T-antigen positive. IC was apparent only within small groups of cells. Maturation of uninfected cells was loosely dependent on cell colony size. Keratinizing cells in infected cultures appeared only after formation of confluent sheets, indicating that SV40 infection greatly increases the cell density-dependence of keratinocyte maturation. The results are consistent with a requirement of IC for differentiation of keratinocytes. (20 Refs)

13. NONLYTIC SIMIAN VIRUS 40-SPECIFIC 100K PHOSPHOPROTEIN IS ASSOCIATED WITH ANCHORAGE-INDEPENDENT GROWTH IN SIMIAN VIRUS 40-TRANSFORMED AND REVERTANT MOUSE CELL LINES.

Chen S, Verderame M, Lo A, Pollack R
Dept. Biological Sciences, Columbia Univ., New York, NY, 10027
Mol Cell Biol; 1(11):994-1006 1981

The two distinct growth controls of normal fibroblasts can be assayed as requirements for serum or anchorage. Interaction of mouse 3T3 fibroblasts with simian virus 40 (SV40) generates four lines of transformed (TR) cells (fully TR, anchorage TR, serum TR and fully reverted). Viral gene expression was studied in these four lines. Immunoprecipitation of (35S)methionine labeled cell extracts with an antiserum from tumor-bearing hamsters detected the SV40 large T and small t proteins (94,000 mol wt [94K], 17K) and the nonviral host 54K protein in all four lines. A 100K tumor antigen was found in some, but not all lines; similar molecules have been reported in many rodent transformed lines. Analysis of the relation of phenotype to relative amounts of the various proteins in the cell classes, using the Spearman rank correlation test, showed the amount of 100K T antigen relative to 94K T antigen or to total viral protein correlated well wi th ability to form colonies in semisolid medium. No significant correlation was found between quantities of labeled 94K T antigen, 54K host antigen, or 17K t antigen and either serum or anchorage independence. Mouse cell TR with small t SV40 deletion mutant 884 synthesized a 100K T antigen, suggesting small t is not required. The 100K T antigen migrated more slowly than the lytic T. It is concluded that since mixtures of extracts from cells expressing and lacking the 100K T antigen yielded the expected amount of this protein, it is unlikely that the 100K T derives from the 94K protein by a posttranslational modification. (70 Refs)

14. INDUCTION OF CELLULAR DEOXYRIBONUCLEIC ACID SYNTHESIS IN BUTYRATE-TREATED CELLS BY SIMIAN VIRUS 40 DEOXYRIBONUCLEIC ACID.

Kawasaki S, Diamond L, Baserga R
(c/o Baserga) Dept. Pathology, Temple Univ. Sch. Medicine, Philadelphia, PA, 19140
Mol Cell Biol; 1(11):1038-1047 1981

The effect of a small DNA oncogenic virus, simian virus 40 (SV40), on sodium butyrate (SB) inhibition of growth was studied in the attempt to explain the mechanisms involved. SB (3 milliM [mM]) inhibited entry into the S phase of quiescent 3T3 cells stimulated by serum, but had no effect on cellular RNA accumulation. SV40 infection or microinjection of cloned fragments from SV40 A gene caused entry of quiescent 3T3 cells into the S phase even in the presence of SB. Cells of an SV40-transformed 3T3 line, NGI, grew vigorously in 3 mM SB and homokaryons were formed between G1 and S-phase 3T3 cells. SB inhibited the induction of the DNA synthesis usually seen in G1 nuclei when G1 cells were fused with S-phase cells. When G1 3T3 cells were fused with exponentially growing NGI cells, 3T3 nuclei entered DNA synthesis. In temperature sensitive (ts) AF8 cells, an RNA polymerase II mutant that stops the cell cycle in the G1 phase, no temporal sequence was demonstrated between SB block and the ts block. Results confirm previous reports that certain virally coded proteins can induce cell DNA synthesis in the absence of cellular functions that are required by serum-stimulated cells. An interpretation of the data on SB inhibition of cell growth is presented. (48 Refs)

15. DEPENDENCE OF INTERFACIAL PROPERTIES OF NORMAL AND TRANSFORMED 3T3 CELL MEMBRANES ON TREATMENT WITH FACTORS MODIFYING PROLIFERATION.

Adam G, Schumann C
Fakultat fur Biologie, Universitat Konstanz, D-7750 Konstanz, W. Germany
Cell Biophys; 3(3):189-209 1981

The interfacial properties of cells after treatment with factors modifying cell proliferation were studied by measuring contact angles. Cell lines used were mouse 3T3 and the DNA-virus-transformed derivatives, SV40-3T3 and PY-3T3. Treatment of normal or transformed cells with 20 or 50% newborn calf serum (NCS) produced a drastic decrease in interfacial tension. This decrease depended on NCS concentra tion and was correlated with the capacity of NCS to induce cell proliferation. Transformed cells were more sensitive to variations in concentration than normal cells. Concanavalin A (ConA) produced the opposite effect, increasing interfacial tension in a concentration-dependent manner; the increase was by a factor of approx 2.6 at a concentration of 50 ug/ml. Succinyl concanavalin A (SConA) produced a smaller effect, requiring approx 500 ug/ml for the same increase. Such results correlated with the lower inhibition of cell proliferation by SConA compared with ConA. No effect on interfacial tension could be established for mitotic inhibitors such as colchicine, possibly due to the intracellular location of their targets. (45 Refs)

16. INTERACTION OF SERUM AND CELL SPREADING AFFECTS THE GROWTH OF NEOPLASTIC AND NON-NEOPLASTIC FIBROBLASTS.

Tucker RW, Butterfield CE, Folkman J
Cell Proliferation Lab., Johns Hopkins Oncology Center, Baltimore, MD, 21205
J Supramol Struct Cell Biochem; 15(1):29-40 1981

The effects of variations in serum concentration and cell spreading on the growth of neoplastic and non-neoplastic cells was studied. Cell cultures of murine non-neoplastic 3T3 and simian virus 40 (SV40)-transformed 3T3 cells, Chinese hamster embryo non-neoplastic CHEF 18-1 and neoplastic CHEF 16-2, cells and human fibrosarcoma HT1080 cells were tested. Cell spreading was controlled with films of poly(2-hydroxyethyl methacrylate) in concentrations of 10(-2)-10(-4) dilutions. Cell numbers grew in proportion to cell spreading in both neoplastic and non-neoplastic cultures. Maintenance of cell rounding inhibited growth of non-neoplastic cultures, but not of neoplastic cultures. Net cell numbers did not increase in some neoplastic cultures when serum concentrations were increased above 10% with increased cell spread. All non-neoplastic cultures showed increased growth with increasing serum concentrations as they converted to the spread form. Sensitivity of normal cells to humoral factors evidently is controlled by cell form (spreading). This control is less evident in at least some neoplastic cells. (20 Refs)

17. ONCOGENIC ACTIVITY OF THE BK TYPE OF HUMAN PAPOVA VIRUS IN NEWBORN WISTAR RATS.

Noss G, Stauch G, Mehraein P, Georgii A
Institut fur Virologie und Seuchenhygiene, Medizinische Hochschule Hannover, Karl-Wiechert-Allee 9, D-3000 Hannover 61, W. Germany
Arch Virol; 69(3/4):239-251 1981

The oncogenic potential of the human papova virus BK (= BKV) has been examined in newborn Wistar rats. Twelve of 37 animals inoculated with BKV sc and 7 of 40 animals inoculated with BKV ic developed tumors of various histological types. The latency periods ranged from 6 to 18 mo. The BKV etiology of tumors was supported by detection of BKV T antigen in cells of established tumor lines by means of indir ect immunofluorescence. No tumors appeared in animals given injections of saline instead of virus. BKV T antibodies were detected in sera of 100% of animals bearing tumors but not in sera of control animals. Only 3 of 77 BKV infected and none of the control animals developed spontaneous tumors (adenomas). (Author abstract) (16 Refs)

18. EFFECT OF HEAT SHOCK ON PROTEIN SYNTHESIS BY NORMAL AND MALIGNANT HUMAN LUNG CELLS IN TISSUE CULTURE.

Tsukeda H, Maekawa H, Izumi S, Nitta K
Dept. Virology, Natl. Inst. Health, Shinagawa-ku, Tokyo 141, Japan
Cancer Res; 41(12):5188-5192 1981

Heat shock proteins were found in cultured human lung cells by sodium dodecyl sulfate:gel electrophoresis and autoradiography using [35S]methionine. The synthesis of a mol wt 70,000 protein was markedly stimulated in normal, malignant, and SV40-transformed cells, and that of mol wt 90,000 and mol wt 100,000 proteins was stimulated only in malignant and SV40-transformed cells after heat shock treatment. In contrast, the synthesis of mol wt 200,000 and mol wt 250,000 proteins observed in the unheated normal cells was diminished after the same procedure. The heat shock proteins were induced within a given range of temperature and duration of treatment, the conditions for normal and malignant cells being clearly different, ie, optima of 43 C for 1 hr in the normal cells and 41 C for 1 hr in the malignant cells. The results suggest that the analysis of heat shock protein is very useful for identifying the differential heat susceptibilities of normal and malignant cells and for elucidating their bases and mechanisms. (Author abstract) (27 Refs)

19. TWO POLYOMA VIRUS GENE FUNCTIONS INVOLVED IN THE EXPRESSION OF THE TRANSFORMED PHENOTYPE IN FR 3T3 RAT CELLS: I. LOCALIZATION OF TRANSFORMATION MAINTENANCE FUNCTION IN THE PROXIMAL HALF OF THE LARGE T CODING REGION.

Rassoulzadegan M, Gaudray P, Canning M, Trejo-Avila L, Cuzin F
(c/o Cuzin) Centre de Biochimie du CNRS, Universite de Nice, Parc Valrose, 06034 Nice, France
Virology; 114(2):489-500 1981

The hypothesis is presented that the expression of a fully transformed phenotype in FR 3T3 rat fibroblast cells transformed with polyoma virus requires two viral gene products. One is the membrane-associated middle T protein (as previously recognized in a number of laboratories). A second necessary function is dependent on an N-terminal region of the large T protein. It can be exerted either by the full-size large T protein (105K), or by truncated forms deleted in the C-terminal region. This hypothesis is supported by the results of a complementation analysis using the tsa mutant, which affects only the complete large T protein, and a cloned segment of the viral DNA that codes only for the N-terminal part of large T (BamHI-EcoRI fragment of an hrt (NG 18) deletion). Results show that this subgenomic fragment is able to complement the tsa defect for the maintenance of the transformed state, but not for its establishment. (Author abstract) (39 Refs)

20. TWO POLYOMA VIRUS GENE FUNCTIONS INVOLVED IN THE EXPRESSION OF THE TRANSFORMED PHENOTYPE IN FR 3T3 RAT CELLS: II. THE PRESENCE OF THE 56K MIDDLE-T PROTEIN IN THE CELL MEMBRANE IS NOT SUFFICIENT FOR MAINTENANCE.

Trejo-Avila L, Gaudray P, Cuzin F
(c/o Cuzin) Centre de Biochimie du CNRS, Universite de Nice, Parc Valrose, 06034 Nice, France
Virology; 114(2):501-506 1981

Rat fibroblast cells transformed with the tsa mutant of polyoma virus (Type N) exhibit a fully transformed phenotype when grown at low temperature and synthesize the three early viral proteins, large T, middle T, and small T. After a shift to the restrictive temperature, these cells revert to the normal phenotype. They still synthesize the middle T protein, undistinguishable from that present in phenotypically transformed cells on the basis of its apparent mol wt, association with cell membrane, and in vitro phosphorylation. Therefore, the synthesis of middle T and its association with the cell membrane do not appear to be sufficient conditions for the expression of the transformed phenotype. (Author abstract) (23 Refs)

21. TRANSFORMATION OF RAT FIBROBLASTS BY CLONED DEFECTIVE POLYOMA DNA.

Parent C, Bastin M
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Arch Int Physiol Biochim; 89(3):225-233 1981

To define the sequence of polyoma (Py) DNA that is essential for cell transformation, defective Py DNA molecules were cloned in plasmid (p) BR322 and the recombinant plasmids were tested for their capacity to transform Fischer rat 3T3 cells in culture. Recombinant plasmids containing an intact proximal portion of the early region were able to transform. All DNA's capable of transforming cells contained an intact 5'-portion of the early region, ie, the region mapping between 70 and 100 map units. pH-df55, the recombinant lacking the DNA sequences extending from 92 to 24 map units, failed to transform. This shows the importance of DNA sequences encoding the C-terminal portion of Py middle T antigen in transformation. The frequency of transformation was of the same order for the three strains of Py virus. Transformation frequency varied little for recombinants containing an intact early region (such as pB4 and pH9) than those carrying only the proximal portion of the early region (such as pPA8). Furthermore, cells transformed by the proximal portion of the early region (PA series) grew as fast as those established by transfection with the complete Py genome (H series). Although the results do not exclude the possibility that small T antigen may play some role in transformation, they indicated that this antigen cannot be responsible for induc ing the expression of the transformed phenotype. (28 Refs)

22. GLYCOPEPTIDES PREPARED FROM MOUSE CEREBRUM INHIBIT PROTEIN SYNTHESIS AND CELL DIVISION IN BABY HAMSTER KIDNEY CELLS, BUT NOT IN THEIR POLYOMA VIRUS-TRANSFORMED ANALOGS.

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Exp Cell Res; 136(1):31-41 1981

Glycopeptides isolated from mouse cerebral cortex cell surfaces (BCSG) were shown to inhibit cell growth and protein synthesis in baby hamster kidney (BHK)-21 cells, whereas polyoma virus-transformed BHK-21 cells (pyBHK-21) were refractory to the inhibitory activity of the glycopeptides. Growth inhibition was shown to be reversible and non-lethal to BHK-21 cells. Despite that difference in sensitivity to the action of the glycopeptides, both cell lines could bind the inhibitor in a saturable fashion and in similar quantities. After trypsinization, BHK-21 cells appeared refractory to the inhibitor, whereas pyBHK-21 cells became sensitive. The data suggested the presence of a receptor for BCSG on the cell surface of both cell lines. Incubating BCSG with conditioned medium from pyBHK-21 cells resulted in loss of the glycopeptide's inhibitory activity. In contrast, medium conditioned by BHK-21 cells had no effect on the inhibitory activity of BCSG. We hypothesize that the refractoriness of pyBHK-21 cells to BCSG is related to their autonomous growth characteristics and failure to respond to topo-inhibitory growth control. BCSG may be a naturally occurring growth regulatory whose function can be explored by use of the BHK-21/pyBHK-21 model system. (Author abstract) (34 Refs)

23. ONCOGENICITY OF HEAT-INACTIVATED SIMIAN ADENOVIRUS SA7.

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Arch Virol; 69(3/4):189-196 1981

Simian adenovirus SA7 heated for 30 min at 70 C retained part of its ability to induce tumors in newborn hamsters. Tumors were also induced by DNA extracted from the heated virus. However, neither residual replicating, ie cytopathogenic, nor transforming and T-antigen-inducing activities could be detected in vitro in CV-1 and hamster embryo cells inoculated with the heated virus. The results are discussed in terms of a possible release of viral nucleic acid at the high temperature. They also show that current tests for effective virus inactivation based exclusively on the recognition of replicating virus should be reevaluated. (Author abstract) (19 Refs)

24. ONCOGENE LOCALIZATION IN DNA OF SIMIAN ADENOVIRUSES. I. IN VITRO TRANSFORMATION OF RAT CELLS WITH SV20 AND ITS DNA.

Parkhomenko II, DenisovaTS, Sitnikov BS, Bogatyrenko TN, Eliseeva LV
Lab. Physico-Chemical Basis of Oncology, Inst. Chemical Physics, Moscow, USSR
Tsitologiia; 23(8):932-936 1981

The results of comparative evaluation of in vitro transformation of kidney and embryo cells of Wistar rats after treatment with complete and incomplete simian adenovirus SV20 are reported. Defective virus provided transformation of 16% of infected colonies, compared with 1% transformation after treatment with complete virus. DNA from SV20 induced fibrosar-

comas in 100% of newborn Syrian hamsters. (11 Refs) (In RUS)

25. LOCALIZATION OF ONCOGENE IN DNA OF SIMIAN ADENOVIRUSES. II. IDENTIFICATION IN SV20 DNA OF SPECIFIC FRAGMENT WITH TRANSFORMATION ACTIVITY.

Parkhomenko II, Denisova TS, Sitnikov BS
Lab. Physicochemical Basis of Oncology, Inst. Chemical Physics, Moscow, USSR
Tsitologiia; 23(9):1026-1030 1981

DNA of simian adenovirus SV20 was subjected to hydrolysis with the specific restriction endonucleases EcoRI, BamHI, XbaI, and HindIII. Transformation activity of DNA fragments produced by the BamHI was similar to that of the intact DNA. Absence of transformation activity in the fragments produced by the EcoRI indicated the left terminal position of the oncogene in the virus genome. (11 Refs) (In RUS)

26. CONSISTENT APPEARANCE OF MICROTUBULES IN CELLS PRODUCTIVELY INFECTED WITH VARIOUS STRAINS OF TYPE 2 HERPES SIMPLEX VIRUS.

Nii S, Yasuda Y, Kurata T, Aoyama Y
Dept. Virology, Okayama Univ. Medical Sch., Shikatacho, Okayama 700, Japan
Biken J; 24(1/2):81-87 1981

Electron microscopic examination showed microtubular structures in FL cells infected with all 18 strains of HSV-2 examined, but not in cells infected with 9 strains of HSV-1. These structures were also detected in other cultured cells (Vero and Earle's L cells) infected with HSV-2, and also in vivo in cells, such as neuronal cells of the spinal ganglia and liver cells, of 1-day-old suckling mice (DDD strain) infected with this type of virus. Thus the microtubules were consistently detected in cells productively infected with HSV-2. No other herpesviruses so far examined produced microtubular structures such as those observed in HSV-2 infected cells. (Author abstract) (12 Refs)

27. MOLECULAR CLONING OF EQUINE HERPESVIRUS TYPE 1 DNA: ANALYSIS OF STANDARD AND DEFECTIVE VIRAL GENOMES AND VIRAL SEQUENCES IN ONCOGENICALLY TRANSFORMED CELLS.

Robinson RA, Tucker PW, Dauenhauer SA, O'Callaghan DJ
Dept. Microbiology, Univ. Mississippi Medical Center, Jackson, MS, 39216
Proc Natl Acad Sci USA; 78(11,part2):6684-6688 1981

Genomic DNA sequences of equine herpesvirus type 1 (EHV-1) have been cloned as BamHI and EcoRI restriction fragments into the plasmid pBR322 and propagated in Escherichia coli. With the exception of two EcoRI restriction fragments that reside in the S region of the viral genome, all of the cloned fragments demonstrated the same electrophoretic mobilities, restriction cleavage sites, an d blot-hybridization patterns as did the parent fragments produced by BamHI or EcoRI digestion of virion DNA. The EcoRI J fragment and the BamHI E fragment of the L-region terminus were cloned after the addition of appropriate linker oligonucleotides. Fragments originating from each of the two isomeric forms of EHV-1 DNA were contained in this library of clones. Supramolar DNA fragments present only in the DNA of defective interfering (DI) particles of EHV-1 were generated from Bgl II digestion of DNA preparations enriched for EHV-1 DI particles and were cloned as Bgl II and EcoRI fragments into the plasmid vector. The cloned viral sequences represented in this defective genome mapped to the S region of EHV-1 DNA. Blot-hybridization analysis of EHV-1 transformed and tumor cell DNAs with the cloned BamHI B fragment confirmed that subgenomic viral sequences are present and indicted that those sequences map to the viral genome between 0.32 and 0.43 map unit. (Author abstract) (22 Refs)

28. CORRELATION OF IN VITRO PROPERTIES AND TUMOURIGENICITY OF HAMSTER KIDNEY CELLS TRANSFORMED BY BK VIRUS.

Possati L, De Luca S, Ciarallo G, Portolani M
Inst. Microbiology, Univ. Ancona, Via Grotte di Posatora, 60100 Ancona, Italy
J Gen Virol; 55(part2):481-486 1981

Hamster kidney cells transformed by BK virus (HKBK cells) were studied at low passage (about 30 subcultures) after transformation and at high passage (about 130 subcultures) after transforming. Several in vitro properties (rescue of virus, presence of T antigen, colony formation on plastic, in soft agar, on monolayers of normal cells, and serum dependency of growth) and tumorigenicity of these cells for newborn and adult hamsters were compared. HKBK cells at low passage showed high levels of T antigen, the growth properties of normal cells and low tumor-producing ability, while HKBK cells at high passage showed low levels of T antigen, the growth properties of transformed cells and high tumor-producing ability. These results may indicate that cellular transformation by BK virus is initiated, but not maintained, by the expression of genes regulating T antigen, and that the maintenance of the transformed state is due to complicated cellular and viral gene interactions occurring during the course of cell life. (Author abstract) (18 Refs)

29. CYTOMEGALOVIRUS CAUSES A LATENT INFECTION IN UNDIFFERENTIATED CELLS AND IS ACTIVATED BY INDUCTION OF CELL DIFFERENTIATION.

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J Exp Med; 154(5):1636-1651 1981

Murine cytomegalovirus (MCMV) productively infected differentiated murine teratocarcinoma (MTC) cell lines, but did not productively infect undifferentiated MTC cell lines. Undifferentiated and differentiated cells bound similar amounts of radioactively labeled virus. In undifferentiated cells replication of infectious virus, viral antigens, or viral RNA did not occur until the cells were induced to differentiate. (51 Refs)

30. INABILITY TO RESCUE VIRAL GENES FROM HUMAN CELLS BIOCHEMICALLY TRANSFORMED BY HERPES SIMPLEX VIRUS TYPE 2 DNA.

Campione-Piccardo J, Rawls WE
Dept. Pathology, McMaster Univ., Hamilton, Ontario L8N 3Z5, Canada
Can J Microbiol; 27(10):1123-1128 1981

Thymidine kinase negative (TK-) mutants of HSV-1 were used to attempt to rescue HSV-2 genes from cells biochemically transformed with HSV-2DNA. The results indicate that the occurrence of a rescue event was less than 7 x 10(-7) in this system. (Author abstract) (31 Refs)

31. THE SITE OF INTEGRATION OF THE HERPES SIMPLEX VIRUS TYPE 1 THYMIDINE KINASE GENE IN HUMAN CELLS TRANSFORMED BY AN HSV-1 DNA FRAGMENT.

Kit S, Hazen M, Otsuka H, Qavi H, Trkula D, Dubbs DR
Div. Biochemical Virology, Baylor Coll. Medicine, Houston, TX, 77030
Int J Cancer; 28(6):767-776 1981

To analyze the site of integration of the herpes simplex virus Type I (HSV-I) thymidine kinase (TK) gene in biochemically transformed human cells, TK- HeLa(BU25) cells were transformed to the TK+ phenotype by a cloned, 2 kbp PvuII fragment of HSV-1 DNA. The transformed cells [HeLa(BU25)/TF pAGO PP3] were fused with mouse LM(TK-) cells, and human-mouse somatic cell hybrid clones (LH PP3 clones 1, 2, 3, 5 and 6) were isolated in HATG-ouabain selective medium. The HeLa(BU25)/TF pAGO PP3 cells and the LH PP3 hybrid clones expressed HSV-1-specific TK activity and a herpesvirus-associated nuclear antigen, and contained herpesvirus nucleotide sequences. Molecular hybridization experiments were carried out to map the HSV-I and flanking cellular nucleotide sequences in the biochemically transformed cells. TK- revertant subclones isolated by growing the LH PP3 clone 5 cells in BrdUrd (and diphtheria toxin) failed to form colonies in HATG medium, but retained HSV-I nucleotide sequences. Isozyme analyses on 21 gene-enzyme systems representing 21 human chromosomes revealed that all of the LH PP3 clonal lines expressed human hexosaminidase B, which has been assigned to chromosome 5, and all were sensitive to diphtheria toxin, which is also a marker for chromosome 5. Chromosome analyses showed that chromosome 5 was the only human chromosome present in mitoses of LH PP3 clone 5 cells and that human chromosome 5 was present in most of the mitoses of LH PP3 clone 1, 2, 3, and 6 cells. The latter clones also contained 1 or 2 additional human chromosomes in some of the cells. As expected from the molecular hybridization analyses, TK- revertants of LH PP3 clone 5 cells retained portions of chromosome 5 and expressed human hexosaminidase B. The results indicate that HSV-I nucleotide sequences were stably integrated in the biochemically transformed cells, most likely in human c hromosome 5. (50 Refs)

32. GENITAL CANCER AND VIRUSES.

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Gynecol Oncol; 12(2,part2):S25-S41 1981

Current data suggesting an association between viruses and genital cancer are reviewed. The main emphasis is on the relationship between herpes simplex virus Type 2 (HSV-2) and cervical cancer. Seroepidemiological studies are presented along with emphasis on HSV-transforming and oncogenic potential. Although the evidence is indirect, it is highly suggestive. HSV-2 has the oncogenic potential to produce neoplastic disease in animals. The ability of HSV-2 to transform cells in vitro and for transformed cells to produce tumors in vivo is presently the most direct evidence for oncogenic potential. In vivo experiments, more closely linking HSV-2 and cervical cancer are hampered by the severe cytopathic effect of HSV-2. Evidence associating two other viruses, human cytomegalovirus (CMV) and human papilloma virus (PV), with cervical and prostatic cancer are also discussed. Factors associating CMV with genital cancer include the establishment of persistent infections of the genitourinary tract, transmission by sexual contact, seroepidemiological studies with antibodies and genital cancer, detection of virus particles or virus-specific antigens in genital cancer biopsies, and in vitro transformation studies. Factors associating PV with genital cancer include association with venereal disease, conversion of condylomata acuminata to malignant state, and detection of genital carcinoma within condylomata. (97 Refs)

33. 'THE HERPESVIRUS HYPOTHESIS' - ARE KOCH'S POSTULATES SATISFIED?

Aurelian L, Manak MM, McKinlay M, Smith CC, Klacsmann KT, Gupta PK
Dept. Comparative Medicine, Johns Hopkins Medical Inst., Baltimore, MD, 21205
Gynecol Oncol; 12(2,part2):S56-S87 1981

The evidence associating herpes simplex virus Type 2 (HSV-2) with squamous cervical carcinoma is critically reviewed. Thirteen criteria for a causal relationship between a virus and a human cancer are listed and evidence is presented demonstrating that in the case of HSV-2 and cervical cancer these have been fulfilled. The original Koch postulates are discussed from the standpoint of modern concepts of virology. In this context, emphasis is placed on the identification in cervical tumor cells of the viral protein ICP 10/AG-4. This protein is expressed in HSV-2 transformed hamster cells and its expression correlates well with the oncogenic potential of the transformed lines. Consistent with these findings, the expression of ICP 10/AG-4 in cervical cells and the prevalence of the specific antibody that it induces, reflect the progression of the cervical tumor. (Author abstract) (92 Refs)

34. MALIGNANT TUMOURS IN TUPAIA (TREE SHREW).

Hofmann W, Moller P, Schwaier A, Flugel RM, Zoller L, Darai G
Pathologisches Institut, Universitat Heidelberg, Im Neuenheimer Feld 220, D-6900 Heidelberg 1, W. Germany
J Med Primatol; 10(2/3):155-163 1981

Nine spontaneous malignant tumors of the tree shrew were detected and analyzed during an observation period of 9 yr. The tumors were histopathologically examined and classified. All tumors developed in imported tupaia only. From the tumor cells of two different animals new tupaia herpesviruses were isolated. This is the first report on spontaneous malignant tumors of tupaia in captivity. (Author abstract) (11 Refs)

VIRUS REPLICATION AND EXPRESSION

35. NUCLEOTIDE SEQUENCES OF 5' TERMINI OF ADENOVIRUS 2 EARLY TRANSFORMING REGION E1A AND E1B MESSENGER RIBONUCLEIC ACIDS.

Hashimoto S, Wold WS, Brackmann KH, Green M
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Biochemistry; 20(23):6640-6647 1981

The major 5'-terminal RNase T1 oligonucleotides derived from human adenovirus type 2 (Ad2) early regions E1a and E1b were characterized and sequenced. Poly(A+) polyribosomal RNA was isolated from Ad2-infected KB cells cultured 10 h in the presence of cycloheximide, the 5'-terminal m7Gppp was removed, and the 5'-OH of the penultimate 2'-O-methylated nucleoside was labeled with [gamma-32P]ATP by using polynucleotide kinase. 5'-32P-labeled poly(A+) RNA was hybridized to EcoRI-A, HpaI-E, and HpaI-C and to cloned fragments representing map positions 0-4.5, 4.5-8, 8-10.7, 8-17, and

17-31.5. Fragment-specific mRNA was digested with RNase T1, the resulting oligonucleotides were resolved by two-dimensional paper electrophoresis-homochromatography, and the 5'-terminal methylated oligonucleotides were characterized by nuclease P1 digestion and two-dimensional thin-layer chromatography. Two major 5'-terminal RNase T1 oligonucleotides were obtained from EcoRI-A-specific mRNA; these were characterized by RNase T2 digestion and two-dimensional thin-layer chromatography and were sequenced by partial nuclease P1 digestion and electrophoresis-homochromatography These two oligonucleotides were mapped to E1a and E1b, respectively, by analysis of E1a- and E1b-specific mRNA. Their sequences are as follows: E1a, m7Gpp(m6)AmC(m)UCUUGp; E1a,m7Gppp(m(6))AmC(m)UCUUGp;
E1b,m7Gppp(m(6))AmC(m)AUCUGp, in which the methylations shown in parentheses are partial. The E1a 5'-terminal oligonucleotide was found as a minor component of E1b mRNA, implying that mRNAs exist with both E1a and E1b sequences. Five other minor 5'-terminal oligonucleotides were observed in E1 mRNA, implying that transcription initiation is not always precise. Sequences corresponding to these two 5' termini were found at nucleotide positions 498-504 (E1a) and 1699-1705 (E1b) in the Ad2 DNA sequence, positions where the 5'-terminal region of E1a and E1b mRNAs has been mapped by nuclease gel analysis and electron microscopic visualization. (50 Refs)

36. ADENOVIRUS DNA REPLICATION IN VITRO: PURIFICATION OF THE TERMINAL PROTEIN IN A FUNCTIONAL FORM.

Enomoto T, Lichy JH, Ikeda JE, Hurwitz J
Dept. Physiological Chemistry, Faculty Pharmaceutical Sciences, Univ. Tokyo, Tokyo 113, Japan
Proc Natl Acad Sci USA; 78(11,part2):6779-6783 1981

The 80,000-dalton form of the adenovirus (Ad) terminal protein (pTP) has been purified from Ad-infected HeLa cells. pTP was assayed by its ability to form a covalent complex with dCMP. The protein copurified with an activity that is essential for in vitro Ad DNA replication (Ad protein activity) as well as with a DNA polymerase activity that was distinguished from those of HeLa cell DNA polymerase alpha, DNA polymerase beta, and DNA polymerase gamma. The Ad protein-associated DNA polymerase activity was detected with activated DNA but not with poly(rA).oligo(dT) as template and was insensitivie to aphidicolin and sensitive to N-ethylmaleimide. The Ad protein, DNA polymerase, and pTP-dCMP complex-forming activities sedimented in a glycerol gradient as a single peak with an apparent molecular size of 180,000 daltons. NaDodSO4/polyacrylamide gel analysis of the glycerol gradient fraction showed major bands of 80,000 and 140,000 daltons. The 80,000-dalton band was identified as pTP by comparison of its tryptic peptide map with that of the 55,000-dalton form of the terminal protein, which was purified from Ad virions. (Author abstract) (23 Refs)

37. LATE NONSTRUCTURAL 100,000-AND 33,000-DALTON PROTEINS OF ADENOVIRUS TYPE 2: I. SUBCELLULAR LOCALIZATION DURING THE COURSE OF INFECTION.

Gambke C, Deppert W
Friedrich Miescher Inst., CH-4002 Basel, Switzerland
J Virol; 40(2):585-593 1981

We analyzed the subcellular locations of the late adenovirus Type 2 nonstructural 100,000-dalton (100K) and 33K proteins in adenovirus Type 2-infected HeLa cells both by biochemical cell fractionation and by immunofluorescence microscopy, using specific antisera against purified sodium dodecyl sulfate-denatured 100K and 33K polypeptides. Both methods showed that the 100K protein was present in the cytoplasm as well as in the nuclei of infected cells and that it accumulated in the nuclei during the course of infection. Phosphorylated 100K protein also was found both in the cytoplasm and in nuclei. However, the nuclear 100K protein pool was phosphorylated to a higher degree than the cytoplasmic pool. In all experiments the 33K protein, which also is a phosphoprotein, was present exclusively in the nuclei of infected cells. The 100K and 33K proteins were associated with different nuclear substructures; this was demonstrated serologically by an analysis of infected cells in which double color immunofluorescence microscop y was used. In these experiments antibodies against the 100K protein decorated different nuclear structures than antibodies against the 33K protein. (Author abstract) (30 Refs)

38. LATE NONSTRUCTURAL 100,000-AND 33,000-DALTON PROTEINS OF ADENOVIRUS TYPE 2: II. IMMUNOLOGICAL AND PROTEIN CHEMICAL ANALYSIS.

Gambke C, Deppert W
Friedrich Miescher Inst., CH-4002 Basel, Switzerland
J Virol; 40(2):594-598 1981

For an immunological analysis of the late adenovirus Type 2 nonstructural 100,000-dalton (100K) and 33K proteins, we prepared antisera against sodium dodecyl sulfate-denatured, gel-purified 100K and 33K proteins. These antisera were tested for potential cross-reactivity, since according to a previous report these two proteins exhibit extensive amino acid homologies. However, immunoprecipitations of 100K and 33K proteins, as well as a sensitive immune replica technique, did not reveal any immunological relationship between these proteins. Therefore, using fingerprint peptide analysis, we investigated the structural relationship between 100K and 33K proteins labeled with a 14C-amino acid mixture or with [14C]proline after digestion with trypsin. We detected only minor, if any, amino acid homologies, indicating that the 100K and 33K proteins are not structurally related. (Author abstract) (21 Refs)

39. LOCALIZATION OF HEXONE-ENCODING GENE IN GENOME OF SIMIAN ADENOVIRUSES.

Vilnis AE, Loparev VN, Zavizion BA, Tarasishin LA, Khil'ko SN, Naroditskii BS, Grodnitskaia NA, Vikhnovich EM, Manykin AA
D. I. Ivanovskii Inst. Virology, Moscow, USSR
Vestn Akad Med Nauk SSSR; (9):88-94 1981

Comparative analysis of oligopeptide and nucleotide sequences of human adenoviruses Ad2 and Ad6 and simian adenovirus SA7 (C-8) showed that hexone-encoding gene is localized in the region of 50-60-unit of genome. (23 Refs) (In RUS)

40. MECHANISM OF ACTIVATION OF EARLY VIRAL TRANSCRIPTION BY THE ADENOVIRUS E1A GENE PRODUCT.

Nevins JR
Rockefeller Univ., New York, NY, 10021
Cell; 26(2,part2):213-220 1981

Results obtained with certain adenovirus host range mutants (dl312 and hr1) have demonstrated that a functional viral E1A gene is required for the accumulation of early viral mRNA. In this report, it is demonstrated that the failure to accumulate early viral mRNA in E1A mutant-infected cells is due to a lack of transcription of the appropriate transcription units. The E1A

gene product appears to enhance transcription of the early transcription units since there is not an absolute requirement for its function. At low multiplicities of infection, transcription from the early transcription units can be detected but at a later time. By increasing the multiplicity of infection, this slow activation of early transcription can be enhanced, even though at this multiplicity all input viruses are still mutant. I t was also found that early transcription could be detected in mutant-infected cells if the virus was added to cells in which protein synthesis was inhibited prior to infection. Thus inhibition of cellular protein synthesis can mimic the function of the E1A gene. It is proposed that the role of the E1A gene product in the activation of early viral transcription is to inactivate a cellular factor that prevents transcriptioa from the other early viral promoters. (Author abstract) (48 Refs)

41. ADENOVIRAL HETEROGENEOUS NUCLEAR RNA IS ASSOCIATED WITH HOST CELL PROTEINS.

Van Eekelen CA, Mariman EC, Reinders RJ, Van Venrooij WJ
Laboratorium voor Biochemie, Katholieke Universiteit Nijmegen, Geert-Grooteplein Noord 21, NL-6525-EZ Nijmegen, The Netherlands
Eur J Biochem; 119(3):461-467 1981

In this study irradiation of intact cells with 254-nm UV light was used to cross-link hnRNA to proteins that are closely associated with it. In this way proteins interacting specifically with hnRNA could be identified excluding the possibility of nonspecific binding of proteins to the RNA during cell fractionation. In uninfected HeLa cells two polypeptides of 41,500 and 43,000 mol wt can be cross-linked very efficiently to hnRNA. Other proteins, with mol wt of 36,000 and 37,000, were covalently linked less effectively. Irradiation of adenovirus-infected cells results in the cross-linking of the same polypeptides to hnRNA. It was found, however, that hnRNA from adenovirus-infected cells contains both viral and host transcripts. Both classes of transcripts are quantitatively associated with the nuclear matrix and can be cross-linked by irradiation with equal efficiency. To determine whether both adenoviral and cellular transcripts are associated with the same proteins, the cross-linked adenoviral hnRNA-protein complexes were isolated by preparative hybridization to adenoviral DNA immobilized on Sepharose. The results show that the purified cross-linked adenoviral hnRNA-protein complexes also contain the host 41,500-Mr and 43,000-Mr proteins as major components. This suggests that in the infected cell adenoviral-specific hnRNA is associated with host proteins and that the structural organization of viral hnRNA-protein complexes in infected cells probably is similar to the organization of host hnRNA in uninfected cells. (Author abstract) (34 Refs)

42. THE STABILITY OF EARLY ADENOVIRUS MRNA IS CONTROLLED BY THE VIRAL 72 KD DNA-BINDING PROTEIN.

Babich A, Nevins JR
Rockefeller Univ., New York, NY, 10021
Cell; 26(3,part1):371-379 1981

H5ts125, a temperature-sensitive mutant of adenovirus type 5, is restricted to the early phase of infection when grown at the nonpermissive temperature. One phenotype of the virus is the overproduction of early viral mRNA at the nonpermissive temperature relative to levels found in wild-type-infe cted cells, although normal levels are found at the permissive temperature. We have analyzed this phenomenon for the production of RNA from two specific early viral transcription units, E1A and E1B. Transcription rates from both of these regions were found to be the same in ts125-infected cells as in wild-type-infected cells at the nonpermissive temperature. However, when the cytoplasmic stabilities of the E1A and E1B mRNAs were measured in wild-type- and ts125-infected cells, it was found that at the nonpermissive temperature, the RNAs were 3 to 5x more stable in a ts125 infection than in a wild-type infection. Since the mutation in ts125 maps to the gene for the 72 kd DNA-binding protein, these results imply that a functional 72 kd protein is required for the rapid turnover of early viral mRNA in wild-type-infected cells, indicating that the abundance of early viral mRNA is controlled by the 72 kd DNA-binding protein. (Author abstract) (54 Refs)

43. THE HIGH AFFINITY BINDING SITE ON POLYOMA VIRUS DNA FOR THE VIRAL LARGE-T PROTEIN.

Gaudray P, Tyndall C, Kamen R, Cuzin F
Centre de Biochimie du CNRS, Universite de Nice, 06034 Nice, France
Nucleic Acids Res; 9(21):5697-5710 1981

In order to map the high affinity binding site for the viral large-T protein on polyoma virus DNA, we have developed an assay which does not require purified protein. It is based on the specific elution of the large-T ATPase activity for calf thymus DNA cellulose by recombinant DNA molecules including known sequences of the viral DNA. Using this assay, a high affinity binding site has been mapped on the early region side of the ori region. Binding requires the integrity of a sequence /AGAGGC/TTCC/AGAGGC/ (nucleotides 49 to 64 in the DNA sequence of the A2 strain). Similar repeats of a PuGPuGGC sequence within less than 20 bases are not found within the viral coding regions, but are strikingly common in the control regions of papovaviruses and other eukaryotic DNAs. (Author abstract) (31 Refs)

44. STRUCTURE OF CHROMATIN AT DEOXYRIBONUCLEIC ACID REPLICATION FORKS: PRENUCLEOSOMAL DEOXYRIBONUCLEIC ACID IS RAPIDLY EXCISED FROM REPLICATING SIMIAN VIRUS 40 CHROMSOMES BY MICROCOCCAL NUCLEASE.

Cusick ME, Herman TM, DePamphilis ML, Wassarman PM
Dept. Biological Chemistry, Harvard Medical Sch., Boston, MA, 02115
Biochemistry; 20(23):6648-6658 1981

Replicating simian virus 40 (SV40) chromsomes were found to be similar to other eukaryotic chromosomes in that the rate and extent of micrococcal nuclease (MNase) digestion were greater with replicating than with nonreplicating mature SV40 chromatin. MNase digestion of replicating SV40 chromsomes, pulse labeled in either intact cells or nuclear extracts, resulted in the rapid release of nascent DNA as essentially bare fragments of duplex DNA (3-7 S) that had an av length of 120 base pairs and were degraded during the course of the reaction. The small nascent DNA fragments released from replicating chromosomes represented prenucleosomal DNA (PN-DNA) from the region of replication forks that encompasses the actual sites of DNA synthesis and includes Okazaki fragments. Predigestion of replicating SV40 chromosomes with both Escherichia coli exonuclease III (3'-5') and bacteriophage T7 gene 6 exonuclease (5'-3') resulted in complete degradation of PN-DNA. This result, together with the observation that isolated PN-DNA annealed equally well to both strands of SV40 restriction fragments, demonstrated that PN-DNA originates from both sides of replication forks. Over 90% of isolated Okazaki frag-

ments annealed only to the retrograde DNA template. The characteristics of isolated PN-DNA were assessed by examining its sensitivity to MNase and single strand specific S1 endonuclease, sedimentation behavior before and after deproteinization, buoyant density in CsCl after formaldehyde treatment, and size on agarose gels. In addition, it was observed that MNase digestion of purified SV40 DNA also resulted in the release of a transient intermediate similar in size to PN-DNA, indicating that a DNA-protein complex is not required to account for the appearance of PN-DNA. These and other data provide a model of replicating chromosomes in which DNA synthesis occurs on a region of replication forks that is free of nucleosomes and is designated as prenucleosomal DNA. (57 Refs)

45. EXPRESSION OF ADENOVIRUS TYPE 2 LATE GENES IN HELA CELLS INFECTED WITH ADENOVIRUS TYPE 2 OR ADENOVIRUS TYPE 2-SIMIAN VIRUS 40 HYBRID VIRUSES.

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Dept. Cell Biology, Baylor Coll. Medicine, Texas Medical Center, Houston, TX, 77030
J Virol; 40(2):610-614 1981

The relative levels of expression of the five 3'-coterminal mRNA families encoded in the 16.4 to 99 map unit transcription unit of adenovirus Type 2 (Ad2) are determined in part by the frequency of utilization of five possible polyadenylation sites. The possibility that polyadenylation frequencies at these sites may be regulated was tested by examining the expression of the fiber (polypeptide IV) gene in cells infected with nondefective (ND) Ad2-simian virus 40 hybrid viruses which express a sixth late mRNA family. Both the relative rate of fiber protein synthesis and the relative abundance of fiber mRNA were normal in cells infected with the hybrid viruses Ad2+ND1, Ad2+ND2, or Ad2+ND4. (Author abstract) (23 Refs)

46. CELL SURFACE T ANTIGEN IN CELLS INFECTED WITH SIMIAN VIRUS 40 OR AN ADENOVIRUS-SIMIAN VIRUS 40 HYBRID, AD2+D2.

Ismail A, Baumann EA, Hand R
McGill Cancer Centre, Dept. Medicine, McGill Univ., Montreal, Quebec H3G 1Y6, Canada
J Virol; 40(2):615-619 1981

Cell surface T antigen, detected by a radioimmune assay that uses 125I-labeled S taphylococcus aureus protein A and antibodies against either authentic T antigen or D2 hybrid T antigen, was found in simian virus 40-transformed and -infected cells and in cells infected with an adenovirus-simian virus 40 hybrid, Ad2+D2. In simian virus 40 lytic infection, the surface T antigen appeared at the same time as the nuclear T antigen. (Author abstract) (22 Refs)

47. KINETICS OF INHIBITION OF PAPOVAVIRUS DNA SYNTHESIS BY SUPERINFECTION WITH ADENOVIRUS 2 AND NON-DEFECTIVE ADENOVIRUS 2-SIMIAN VIRUS 40 HYBRID VIRUSES.

Patch CT, Howley PM, Hauser J, Levine AS
Section Infectious Disease, Pediatric Oncology Branch, NCI, NIH, Bethesda, MD, 20205
J Gen Virol; 55(part2):355-365 1981

Simian Virus 40 (SV40) DNA synthesis is inhibited in monkey cells by superinfection with adenovirus 2 (Ad2) and various non-defective Ad2-SV40 hybrid viruses. Similarly, BKV (a human papovavirus) DNA synthesis is inhibited in human cells by superinfection with Ad2. Kinetic studies indicate that inhibition begins during the early phase of the Ad2 lytic cycle. Superinfection with Ad2 does not significantly alter the formation of SV40 T antigen. Superinfection with Ad2 late in the SV40 lytic cycle is less efficient in the inhibition of SV40 DNA synthesis, and the onset of Ad2 DNA synthesis is delayed, compared to superinfection early in the SV40 lytic cycle. These findings suggest that the Ad2 and SV40 genomes may compete to bind an early Ad2 protein which is essential for Ad2 replication, but which blocks SV40 replication. (Author abstract) (28 Refs)

48. REPAIR OF PSORALEN-TREATED DNA BY GENETIC RECOMBINATION IN HUMAN CELLS INFECTED WITH HERPES SIMPLEX VIRUS.

Hall JD, Scherer K
Dept. Cellular and Developmental Biology, Univ. Arizona, Tucson, AZ, 85721
Cancer Res; 41(12):5033-5038 1981

Herpes simplex virus type 1 was treated with 4,5',8-trimethylpsoralen (psoralen) plus near-UV light in order to produce lesions (monoadducts and DNA cross-links) in the viral DNA. Human fibroblasts were infected by damaged virus under conditions in which either a single virus particle or several particles entered a given cell, and the fraction of virus-producing cells was determined. This fraction was significantly greater for multiply infected cells than for singly infected cells, indicating that the psoralen lesions are repaired more efficiently in the presence of homologous, damaged DNA (multiplicity reactivation). Evidence is presented that herpes simplex virus may code for functions which participate in its own repair, both during multiplicity reactivation and during repair which occurs in singly infected cells: (a) host cells deficient in repair of lesions induced by psoralen (xeroderma pigmentosum) or the DNA cross-linking agent mitomycin C (Fanconi's anemia) exhibited normal levels of multiplicity reactivation of psora len-treated herpes virus; (b) while xeroderma pigmentosum cells have been previously shown to be deficient in repair of psoralen-treated adenovirus under conditions of single infection, herpes virus is repaired at near normal levels in these same cells. Recombination levels between genetically marked pairs of herpes viruses were found to increase after treatment of the parental viruses with psoralen, suggesting that psoralen damage stimulates genetic recombination. This stimulation provides convincing evidence for a repair pathway in which genetic recombination between damaged viral genomes can lead to the production of viable virus. (Author abstract) (28 Refs)

49. INHIBITION OF PURIFIED HUMAN AND HERPES SIMPLEX VIRUS-INDUCED DNA POLYMERASES BY 9-(2-HYDROXYETHOXYMETHYL)GUANINE TRIPHOSPHATE. EFFECTS ON PRIMER-TEMPLATE FUNCTION.

Derse D, Cheng YC, Furman PA, StClair MH, Elion GB
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J Biol Chem; 256(22):11447-11451 1981

The inhibition of highly purified herpes simplex virus (HSV)-induced and host cell DNA polymerases by the triphosphate form of 9-(2-hydroxyethoxymethyl)guanine (acyclovir; acycloguanosine) was examined. Acyclovir triphosphate (acyclo-GTP) competitively inhibited the incorporation of dGMP into DNA, catalyzed by HSV DNA polymerase; apparent Km and Ki values of dGTP and acyclo-GTP were 0.15

uM and 0.003 uM, respectively. HeLa DNA polymerase alpha was also competitively inhibited; Km and Ki values of dGTP and acyclo-GTP were 1.2 uM and 0.18 uM, respectively. In contrast, HeLa DNA polymerase beta was insensitive to the analogue. The 'limited' DNA synthesis observed when dGTP was omitted from HSV or alpha DNA polymerase reactions was inhibited by acyclo-GTP in a concentration-dependent manner. Prior incubation of activated DNA, acyclo-GTP, and DNA polymerase (alpha or HSV) resulted in a marked decreased in the utilization of the primer-template in subsequent DNA polymerase reactions. This decreased ability of preincubated primer-templates to support DNA synthesis was dependent on acyclo-GTP, enzyme concentration, and the time of prior incubation. Acyclo-GMP-terminated DNA was found to inhibit HSV DNA polymerase-catalyzed DNA synthesis. Kinetic experiments with variable concentrations of activated DNA and fixed concentrations of acyclo-GMP-terminated DNA polymerase revealed a noncompetitive inhibition of HSV-1 DNA polymerase. The apparent Km of 3'-hydroxyl termini was 1.1 x 10(-7) M, the Kii and Kis of acyclo-GMP termini in activated DNA were 8.8 x 10(-8) M and 2.1 x 10(-9) M, respectively. Finally, 14C-labeled acyclo-GMP residues incorporated into activated DNA by HSV-1 DNA polymerase could not be excised by the polymerase-associated 3',5'-exonuclease activity. (Author abstract) (15 Refs)

50. LAMELLAR STRUCTURES FOUND IN CELLS INFECTED WITH HERPES SIMPLEX VIRUS TYPE 1.

Oda H, Hirakawa K, Maekawa M, Hayashi Y, Mori R
Dept. Bacteriology, Sch. Medicine, Kagoshima Univ., Usuki, Kagoshima, Kagoshima 890, Japan
Microbiol Immunol; 25(9):959-963 1981

Lamellar structures (LS) were observed in nuclei of cells infected with the KOS strain of herpes simplex virus-1 (HSV-1). Morphological characteristics of the LS were described. Effects of some antimetabolites on the production of the structure were also studied to compare their nature with that of other structures found in cells infected with HSV-1 or HSV-2. Results suggest that LS have properties which are different from those of the intranuclear tubular structures. Nature of the LS is yet to be defined. (7 Refs)

IMMUNOLOGICAL STUDIES

51. MONOCLONAL ANTIBODIES AGAINST ADENOVIRUS TYPE 5: PREPARATION AND PRELIMINARY CHARACTERIZATION.

Russell WC, Patel G, Precious B, Sharp I, Gardner PS
Div. Virology, Natl. Inst. Medical Res., Mill Hill, London NW7 1AA, England
J Gen Virol; 56(part2):393-408 1981

Eight different hybridoma cell lines producing monoclonal antibodies against the major antigens of human adenovirus Type 5 have been obtained. They were selected by screening initial hybridomas by the fluorescent antibody technique followed by radioimmune precipitation and they reacted with hexon, penton, fiber and 100K polypeptides. Five apparently different epitopes against the hexon antigen were detected showing a spectrum of activity against the hexons of other serotypes, suggesting that the hexon contained a variety of subgroup specificities as well as the previously described group and type specificities. (Author abstract) (26 Refs)

52. MINOR HISTOCOMPATIBILITY ANTIGENS DO NOT ENHANCE BALB/C ANTI-SV40 TASA RESPONSE.

Nanni P, De Giovanni C, Galli MC, Grilli S, Lollini PL, Nicoletti G, Prodi G
Istituto di Cancerologia, Via S. Giacomo 14, I-40126 Bologna, Italy
Br J Cancer; 44(4):588-591 1981

Possible enhancement of the immune response to simian virus-40 (SV40)-induced tumor-associated surface antigens (TASA) was studied in major histocompatibility complex (MHC)-matched mice after immunization with TASA along with minor histocompatibility antigens (as adjuvant antigens). An in vitro cytotoxicity test using BALB/c mouse spleen cells immunized with cell lines differing at multiple minor histocompatibility loci (either the syngeneic cell line KB/cSV or the KD2SV cell line) did not detect an adjuvant effect of the minor histocompatibility antigens. In the Winn neutralization assay, 9-10 wk-old female mice were immunized sc with 10(6) KD2SV, KB/cSV, or mKSA-Tu5 cells. Twenty days later, spleen cells from 2-3 animals were pooled, mixed with 10(5) freshly harvested mKSA(ASC) cells at different ratios (25:1, 50:1, 10 0:1), suspended in 0.2 ml phosphate buffered saline, and injected sc into the left hind legs of 11-12 wk-old BALB/c females. Survival of tumor-free animals failed to indicate an adjuvant effect by minor histocompatibility antigens. Both tests failed to detect enhancement of the immune response against SV40-transformed syngeneic cells in BALB/c mice who are immunized with H-2 matched SV40-transformed cells differing at multiple minor histocompatibility loci. (16 Refs)

53. INDUCTION OF TARGET ANTIGENS AND CONVERSION TO SUSCEPTIBLE PHENOTYPE OF NK-CELL-RESISTANT LYMPHOID CELL LINE.

Clark EA, Sturge JC, Falk LA
Regional Primate Res. Center, Univ. Washington, Seattle, WA, 98195
Int J Cancer; 28(5):647-654 1981

Two autologous Herpesvirus papio producer lymphoid cell lines and one autologous non-producer line were compared for susceptibility to natural killer (NK) cell-mediated lysis. The non-producer cell line, 26CB-1, was more resistant to NK cell killing compared to one viral producer counterpart 13CB-I, but equally resistant when compared to another, 8CB-I. Treatment with chemical agents that affect differentiation or activate the viral cycle, including n-butyrate, IuDR, 5-azacytidine and tunicamycin, increased the susceptibility to killing of the non-producer line but had less effect on the 13CB-I producer line. The increase in susceptibility was due to induction of new target antigens: activated 26CB-I cells were more effective at inhibiting NK-cell-mediated lysis and were bound by more NK cells than untreated control cells. The expression of NK target structures may be related to the differentiated state rather than to the viral production status of target cells. (Author abstract) (43 Refs)

54. PREPARATION OF STABLE TARGET CELLS FOR ANTI-HERPES SIMPLEX VIRUS CYTOTOXIC T LYMPHOCYTES.

Sonoda S, Hitsumoto Y, Utsumi S, Takami S, Oseto M, Minamishima Y
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Microbiol Immunol; 25(10):999-1010 1981

With the use of SKa, an avirulent strain of herpes simplex virus (HSV), and a methylcholanthrene-induced mouse sarcoma cell line (Meth A cells), a reliable target cell system was

developed for detection of cell-mediated cytotoxicity directed against HSV-infected cells. Also discussed are the assessment of improved measurement for HSV-specific cytotoxic T lymphocytes (CTL) generated in BALB/c mice, and the practical value of avirulent HSV for the study of cell-mediated immunity against HSV. SKa propagated in Vero cells did not replicate in Meth A cells when infected at multiplicity of infection (moi) 3, whereas strain KOS at the same moi replicated in these cells as well as in Vero cells. Generation of HSV-antigen by SKa advanced so slowly that it reached the max level at 18 hr post-infection (pi) while KOS infection r esulted in a rapid production of HSV antigens which was accomplished within 6 hr pi and followed by abrupt response of cytolysis. SKa-infected Meth A cells released no more 51Cr than noninfected control cells, whereas KOS-infected cells induced an abrupt increase in 51Cr-release at around 8 hr pi. SKa-infected Meth A targets were specifically lysed by immune spleen cells stimulated with HSV-infected Meth A antigens, but not by spleen cells treated with noninfected Meth A cells alone. Thy-1 antigen positive cells are responsible for HSV-specific cytotoxicity. The system described should be valuable in studies of cell-mediated cytotoxicity directed against HSV-infected cells. (42 Refs)

55. MULTIPLE GROWTH PARAMETERS OF LYMPHOID CELL LINES TRANSPLANTED INTO NUDE MICE (MEETING ABSTRACT).

Kirchner HH, Gunzel M, Schaadt M, Fonatsch C, Diehl V

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Exp Hematol; 9(Suppl9):88 1981

Tumorigenic potential of human hematopoetic cell lines in nude mice is not a marker of malignant cells only. To evaluate differences of the growth behavior of diploid, Epstein-Barr virus-transformed lymphoblastoid cell lines (LCL) considered to be non-neoplastic and malignant lymphoma lines (LL) three transplantation routes were examined: intracerebral (ic), sc/im inoculation of single cells suspensions and the sc transplantation of cells embedded in a fibrin clot. Additional to the tumor growth in the different modes of transplantation various parameters were analyzed: latency period, tumor progression, serial transplantability, in vitro reculture success of nude mouse tumors. After intracerebral (ic) inoculation both types of lines were able to induce tumors with a rate of nearly 100%. Generally the LCL had a longer latency period and the rate of tumors which were macroscopically visible was only half of that compared to the lymphoma lines. Serial transplantation showed a constant latency period of tumor appearance with LL whereas with LCL there was an increasing interval between inoculation and tumor formation leading to a failure of tumor induction in a higher passage number. After sc/im transplantation of suspension cells only LL and old LCL with abnormal karyotypes were tumorigenic. The tumor growth curves showed an unlimited progression with similarity to the Gompertz function. When the cells were embedded in a fibrin clot before sc transplantation even diplloid LCL were able to induce tumors, but after initial exponential enlargement these nodules showed a tendency to regression and failed to grow in vitro after recultivation attempts as that stage. The in vitro recultivation of LCL induced tumors at the phase of logarithmic progression resulted in diploid cell lines. (no Refs)

OTHER RELATED STUDIES

56. DIFFERENTIAL EFFECT OF IMIDAZOLE ANTIBIOTICS ON UNTRANSFORMED AND VIRUS-TRANSFOR MED RAT CELL LINES.

Kuwano M, Akiyama S, Kaneko M, Ikezaki K, Takaki R, Kimura G

Dept. Biochemistry and Internal Medicine, Oita Medical Sch., Hazama-cho, Oita 879-56, Japan

Cancer Res; 42(1):280-284 1982

The imidazole antimycotics clotrimazole and miconazole were tested on untransformed rat cell line 3Y1-B clone 1-6 (3Y1) and six transformed cell lines, which were independently isolated from 3Y1 or 3Y1-B clone 1 after infection with adenovirus 12 (AD-12) or with SV40, to determine their sensitivities to these drugs. The relative plating efficiency of three cell lines (T3, W4, and W5) transformed with AD-12 was reduced to 10(-1) of the initial value by clotrimazole (2 to 4 ug/ml), whereas that of the parental cell line 3Y1 was reduced to 10(-1) of the initial value by clotrimazole (20 to 25 ug/ml). By contrast, the differential effect of miconazole on 3Y1 and AD-12-transformed cell lines was found to be a factor of 2. The sensitivity of the SV40-transformed cell lines to these drugs was between the untransformed 3Y1 and the AD-12-transformed cell lines. The cellular sensitivity of untransformed 3Y1 cells to clotrimazole was significantly enhanced when exposed to various doses of the unsaturated fatty acid, linoleic acid. The untransformed and transformed cell lines showed sensitivities similar to the cytocidal activity of sterol-binding antimycotics, amphotericin B, and filipin. (Author abstract) (30 Refs)

57. ANTITUMOR ACTIVITY OF 5-HYDROXYMETHYL-2-FUROIC ACID.

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Agric Biol Chem; 45(9):2149-2150 1981

The compound 5-Hydroxymethyl-2-furoic acid (65-I) has been isolated from the ethylacetate extract of the cultured broth and mycelium of Pyricularia grisea IAM 5051. The compound was shown to have selective cytotoxicity against Simian virus 40 (SV40)-transformed cells in vitro and antitumor activity against Sarcoma 180 in vivo. The isolation procedure and physical characteristics of 65-I are described. The compound had selective toxicity against SV40-transformed C3H-2K cells from day 1 at concentrations from 125 to 2,000 ug/ml; the minimum cytotoxic concentration increased with treatment time. The total packed cell vol (TPCV) method was used to assay antitumor activity in vivo. Ascitic tumor cells of sarcoma 180 were implanted ip in ICR mice (5 wk old, 17.5-20 g). Saline soln of the samples was administered 1x/day for 5 consecutive days (50 mg/kg x 5). The vol of tumor cells in ascites was measured on day 7. The ratio of TPCV of the test group to that of the control group was 36.6; antitumor activity was judged effective. (2 Refs)

58. INHIBITION OF CELLULAR AND VIRUS-ASSOCIATED NUCLEOTIDE POLYMERASES BY, AND ANTI-HERPES SIMPLEX VIRUS ACTIVITY OF, STREPTOVARICIN DERIVATIVES.

Srivastava BI, DiCioccio RA, Chadha KC, Rinehart KL
Dept. Experimental Therapeutics, Roswell Park Memorial Inst., Buffalo, NY, 14263
Antimicrob Agents Chemother; 20(5):653-659 1981

Fourteen streptovaricin derivatives were tested for inhibition of cellular nucleotide polymerases (DNA polymerase alpha, DNA polymerase beta, and DNA polymerase gamma, terminal deoxynucleotidyltransferase [TdT], and RNA polymerase II), simian sarcoma virus deoxyribonucleic acid polymerase, and herpes simplex virus Type 1-induced deoxyribonucleic acid polymerase (HSV-DP). Three compounds (streptovadienal C, prestreptovarone, and streptoval Fc) preferentially inhibited TdT and HSV-DP over the other enzymes. These compounds inhibited HSV-DP more potently than they inhibited TdT. Evidence indicated that the mode of inhibition of TdT and HSV-DP by streptovadienal C and prestreptovarone was by interaction with the enzymes and not with template-primer, initiator, substrates, or divalent cations required for enzyme activity. Furthermore, data suggested that these compounds bind with greater affinity to HSV-DP than to TdT. Streptovadienal C and prestreptovarone were examined for their effect on the replication of herpes simplex virus Type 1 in African green monkey kidney (CV1) cells. These compounds produced 2-and 3-log drops in virus titer, respectively, at concentrations not significantly affecting cell viability. This correlated with evidence indicating a greater binding affinity of these compounds for HSV-DP over cellular nucleotide polymerases. (Author abstract) (27 Refs)

59. DIFFERENTIAL INHIBITION OF HERPES SIMPLEX VIRUSES, TYPE 1 (HSV-1) AND TYPE 2 (HSV-2), BY (E)-5-(2-X-VINYL)-2'-DEOXYURIDINES.

de Clercq E, Verhelst G, Descamps J, Bergstrom DE
Rega Inst. Medical Res., Minderbroedersstraat 10, B-3000 Leuven, Belgium
Acta Microbiol Acad Sci Hung; 28(3):307-312 1981

Antiviral activities of six (E)-5-(2-X-vinyl)-2'-deoxyuridines were tested in primary rabbit kidney cell cultures infected with laboratory strains of herpes simplex virus types 1 and 2. Four of the compounds may be useful as marker tests for rapid and specific diagnosis of human HSV-1 and HSV-2 infections. The tests may be less time-, labor- and reagent-consuming than many previously described typing techniques, including restriction endonuclease mapping. (15 Refs)

60. MURINE TERATOCARCINOMA: A MODEL FOR VIRUS-CELL INTERACTION IN A DIFFERENTIATING CELL SYSTEM.

Friedrich TD, Lehman JM
Jackson Lab., Bar Harbor, ME, 04609
J Supramol Struct Cell Biochem; 15(2):205-211 1981

Infection of murine teratocarcinoma stem cells with simian virus 40 (SV40) was attempted. Cells were cultured from embryonal carcinoma lines 247DESCl2, which originally produced various differentiated cells, and PCC4azal, which can be chemically induced to differentiate. Cell cultures were e xposed to SV40. Infection with SV40 was determined by measuring production of T antigen by immunoprecipitation. No infection could be detected in either 247DESCl2 or PCC4azal cells. Attempts to produce infection and consequent appearance of T antigen included multiple infections of different pools of non-plaque-purified SV40, exposure of cell cultures to 100 ug diethylaminoethyl-dextran/ml for 45 min, pretreatment with 4-nitroquinoline-1-oxide for 24 hr, and coinfection with adenovirus. Measurement of 5-bromo-2'-deoxyuridine uptake indicated no detectable synthesis of viral DNA in PCC4azal cells. (24 Refs)

61. EFFECTS OF ORAL TREATMENT WITH ACYCLOVIR AND BROMOVINYLDEOXYURIDINE ON THE ESTABLISHMENT AND MAINTENANCE OF LATENT HERPES SIMPLEX VIRUS INFECTION IN MICE.

Field HJ, De Clercq E
Dept. Pathology, Univ. Cambridge, Cambridge, England
J Gen Virol; 56(part2):259-265 1981

Mice infected with herpes simplex virus (HSV) were treated (separately) with the nucleoside analogues acyclovir or bromovinyldeoxyuridine by incorporating the drugs in the drinking water. This method of treatment was found to be effective for both drugs and compared favorably with ip injection. Prompt treatment with either compound could prevent the establishment of latent infections but latent infections once established were intractable using prolonged courses of oral administration. (Author abstract) (23 Refs)

62. EFFECT OF HERPES INFECTION ON INDUCTION OF CERVICAL CANCER BY METHYLCHOLANTHRENE IN MICE (MEETING ABSTRACT).

Nishiura H, Nii S, Nasu K
Department of Obstetrics and Gynaecology, Hyogo Medical College, Nishinomiya, Japan
Obstetrics and Gynaecology: 8th Asian Congress, October 25-31, 1981, Melbourne, Australia. Asia and Oceania Federation of Obstetricians and Gynaecologists 352 pp., 1981.

It is unknown whether herpes simplex virus type 2 (HSV-2) is one of the possible etiologic agents of human cervical cancer or not. The present study was performed to evaluate any possible carcinogenic activity of HSV-2 in the genital tract of the female mouse. The 'string method' of methylcholanthrene (MC) was used to induce carcinoma of the uterine cervix in mice. Two kinds of experiments were designed as follows: (1) Impregnation of MC threads was first carried out, and 7 wk later these were removed and half of the mice were infected with HSV-2 in the genital tract, while the other half were not. One, 2 and 3 mo later, several mice from both groups were sacrificed and the histological changes in the squamous and columnar epithelium were examined. A synergistic effect of MC and HSV-2 on the columnar areas was not observed, whereas this effect was clearly demonstrated on the squamous areas, especially in the specimens prepared from the mice at 3 mo after HSV infection. (2) Mice were first infected and 10 days later MC treatment was carried out. Three experimental groups of HSV(+)MC(+), HSV(+)MC(-), and HSV(-)MC(+) treated mice studied as controls. Four mo after MC treatment, all mice were sacrificed and examined. In this experiment, the appearance of atypia III (corresponding to carcinoma-in situ in the human) and invasive lesions of the cervix were frequently detected in the group of HSV(+)MC(+). From these investigations, it is postulated that herpetic infection may enhance induction of cervical cancer. (no Refs)

63. THE MECHANISM OF ACTION OF PLATINUM-CONTAINING ANTITUMOR DRUGS: DNA INTERACTIONS.

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Dies Abstr Int [B]; 42(3):911-B 1981

The mechanism of action of the potent antitumor drug cis-dichlorodiammineplatinum(II) (cis-DDP) has been examined. Maxam-Gilbert Nucleotide sequencing and DNase footprinting demonstrate that cis-DDP binds to sequential guanine sequences on one strand of a DNA restriction enzyme fragment from the origin of replication of a bacterial plasmid. The clinically inactive trans isomer exhibits no sequence specificity. This is consistent with the proposed mechanism of action whereby cis-DDP causes intrastrand crosslinking of adjacent guanine residues. Because of its geometry, the trans isomer cannot participate in an analogous intrastrand crosslinking reaction. Agarose gel electrophoresis of platinum-DNA complexes demonstrates that cis-DDP binds to DNA by two distinct modes, whereas trans-DDP binds by only one mode. These experiments establish, in addition, that cis-DDP promotes unwinding of the duplex. Digestion with the single-strand specific endonuclease S1 reveals that single-stranded regions are concomitantly generated. SV40 large Tumor antigen (TAg) is required to initiate SV40 DNA replication; this protein binds specifically to the unique origin of replication of SV40 DNA. DNase protection experiments demonstrate that cis-DDP inhibits the specific binding of SV40 TAg to the origin of replication of SV40 DNA. These studies indicate that although both cis- and trans-DDP bind to DNA, only cis-DDP binds by a mechanism consistent with intrastrand crosslinking of adjacent guanine bases. This cis-DDP-specific reaction rapidly changes the physical characteristics of the DNA. These perturbations in DNA structure inhibit the interaction between a virus-coded protein required for DNA replication and the modified viral DNA template. (no Refs)

64. THE PRESENCE OF SV40 T-ANTIGEN IS NOT REQUIRED FOR CYTOCHALASIN B-INDUCED MULTINUCLEATION.

Connor CG, Brownstein BL
Dept. Biology, Temple Univ., Philadelphia, PA, 19122
Cell Biol Int Rep; 5(11):1027-1031 1981

Two different rat liver epithelial cell lines sensitive for SV40 transformation were studied to determine if T-antigen is responsible for the uncontrolled nuclear division found in such ce lls treated with cytochalasin B. Since multinucleation was found at temperatures at which T-antigen was not immunologically detectable, T-antigen is not necessary for cytochalasin B-induced multinucleation. (15 Refs)

65. CORE (2'-5')OLIGOADENYLATE AND THE CORDYCEPIN ANALOG: INHIBITORS OF EPSTEIN-BARR VIRUS-INDUCED TRANSFORMATION OF HUMAN LYMPHOCYTES IN THE ABSENCE OF INTERFERON.

Doetsch PW, Suhadolnik RJ, Sawada Y, Mosca JD, Flick MB, Reichenbach NL, Dang AQ, Wu JM, Charubala R, Pfleiderer W, Henderson EE
(c/o Suhadolnik) Dept. Biochemistry, Temple Univ. Sch. Medicine, Philadelphia, PA, 19140
Proc Natl Acad Sci USA; 78(11,part2):6699-6703 1981

The 3'-deoxyadenosine (cordycepin) analog of (2'-5')oligo(A) [(2'-5')oligoadenylate with a triphosphate at the 5' end], synthesized enzymatically from cordycepin 5'-triphosphate in lysed rabbit reticulocytes or L-cell extracts was (i) inhibitory to translation in lysed rabbit reticulocytes and (ii) metabolically stable in extracts of either L cells or C85-5C lymphoblasts. The 5' dephosphorylated (core) (2'-5')oligo(A) and the core cordycepin analog can replace human fibroblast interferon in preventing the transformation of human lymphocytes after infection with Epstein-Barr virus B95-8 (EBV) as determined by the decreased incorporation of [3H]thymidine into cellular DNA and the inhibition of morphological transformation of EBV-infected lymphocytes. Whereas the naturally occurring core (2'-5')oligo(A) was cytotoxic to uninfected lymphocytes and proliferating lymphoblasts, the core cordycepin analog was not. Human WBC interferon was more effective than human fibroblast interferon in the inhibition of EBV-induced transformation of human umbilical cord lymphocytes and adult peripheral blood lymphocytes. (Author abstract) (53 Refs)

66. EFFECT OF 1,4-DIHYDROXY-5,8-BIS (2-(2-HYDROXYETHYLAMINO)ETHYL AMINO)-9,10-ANTHRACENEDIONE (DIHYDROXYANTHRAQUINONE) ON THE REPLICATION OF SIMIAN VIRUS 40 CHROMOSOME.

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Dept. Microbiology, Univ. Kansas, Lawrence, KS, 66045
Biochem Biophys Res Commun; 103(1):249-255 1981

Dihydroxyanthraquinone (DHAQ) is an aminoanthraquinone derivative with potential anticancer activity. While SV40 infection of both permissive and nonpermissive cells stimulates cellular DNA synthesis, the presence of 1.6 nanoM DHAQ inhibited by approx 50% both cellular and viral DNA synthesis. Nearly 90% inhibition was observed at DHAQ concentrations of 0.4 uM or higher. Both RNA and protein synthesis was inhibited 50% in the presence of 0.8-8 uM DHAQ, where DNA synthesis is inhibited by 90%. Results indicate that both cellular and viral chromosome replication are inhibited to the same extent in cells treated with low concentrations of DHAQ. No detectable change in sedimentation on sucrose gradients was observed in viral chromosomes isolated either from DHAQ-treated cells or incubated directly with DHAQ at 2-10 uM. Aggregation of viral chromosomes occurred when very high DHAQ concentrations were used (greater than 20 uM). (21 Refs)

67. THE WAVELENGTH DEPENDENCE OF THE EFFECT OF 8-METHOXYPSORALEN PLUS ULTRAVIOLET RADIATION ON THE INDUCTION OF LATENT SIMIAN VIRUS 40 FROM A MAMMALIAN CELL.

Moore SP, Coohill TP
Dept. Biology, Univ. Louisville, Louisville, KY, 40208
Photochem Photobiol; 34(5):609-615 1981

Combined treatment with 8-methoxypsoralen (8-MOP: 25 ug/ml) and UV in the region 300-365 nanometer induced up to 1,000-fold more infectious simian virus 40 (SV40) virions in SV40-transformed Syrian hamster kidney cells than did treatment with UV alone. In view of the hypothesis that mammals may harbor latent virus and the observation of increased skin cancer in patients given combination treatment with psoralen and UV, 8-MOP plus UV phototherapy should be used with caution. (22 Refs)

68. A NEW SCREENING PROCEDURE ON SELECTIVE INHIBITORS AGAINST SV40-TRANSFORMED CELLS.

Munekata M, Takatsuki A, Onodera K, Tamura G
Res. and Development Lab., Sapporo Breweries LTD., Mita, Meguro-ku, Tokyo 153, Japan
Agric Biol Chem; 45(8):1843-1854 1981

An in vitro assay method of cytotoxicity (N/T assay) and the basic data for N/T assay with several well-known antitumor agents are described. When six compounds were tested in parallel, WIRL-3 epithelial cells and their Simian virus 40 (SV40)-transformed counterparts were more sensitive than C3H-2K fibroblasts and SV40-transformed C3H-2K cells. Patterns of drug sensitivity were different between C3H-2K and SV40-C3H-2K cells. C3H-2K and SV40-C3H-2K cells were used for subsequent experiments. In the method adopted as the standard experimental procedure (namely N/T assay), cells were seeded at 1-2 x 10(4) per one well, and after 20-24 hr of incubation, the medium was replaced with a fresh drug-containing medium. After 72 hr of continuous exposure to the drug, the cells were washed with fresh medium, and refilled with a fresh medium without drugs. The inhibitory effect of well-known antitumor agents having different mechanisms of action are described. The results of testing 61 compounds with/without antitumor activity indicated that antitumor agents with the exception of antimetabolites do not have the selective cytotoxicity generally found in vitro. The pattern of selective cytotoxicity in vitro was classified into five groups by the N/T assay. The antimetabolites belonging to the Group I showed continuous and higher selective cytotoxicity. Bleomycin, oligomycin, and rifampicin, members of Group II had continuous and middle selective cytotoxicity. Group III consisting of energy transfer inhibitors and tunicamycin showed a specific pattern of cytotoxicity, ie, high selective cytotoxicity on day 1, none after day 2. Group IV, including actinomycin D, chromomycin, and cycloheximide, showed middle selective cytotoxicity on day 1, but none after day 2. Group V, including vinblastine, colchicine, mitomycin C, and many antitumor agents, had no selective cytotoxicity even at low concentrations. (20 Refs)

69. REDUCTION OF SIMIAN VIRUS 40 GROWTH BY A MITOTIC INHIBITOR EXTRACTED FROM LIVER. EVIDENCE THAT THE INHIBITOR IS ARGINASE.

Campadelli-Fiume G, Scannavini M, Falasca A, Hakim G, Busi C, Mattioli A, Fiume L
Istituto di Microbiologia e Virologia, Universita di Bologna, via San Giacomo 12, 40126 Bologna, Italy
Ann Sclavo; 23(2):162-168 1981

The effect of liver preparation was studied on the growth of SV 40 in CV1 cells, trying to establish whether the inhibitory effect was due to enzymatic destruction of one of the amino acids, present in the culture medium, and essential for cell DNA synthesis and growth. The mitotic inhibitor was extracted from rat liver and prepared as reported before. Max inhibition was obtained with 50 ug liver preparation protein/ml medium. When only the liver preparation and medium were incubated arginine disappeared and ornithine appeared, which suggest that liver preparations contain the enzyme arginase, which transforms arginine into ornithine. The addition of arginine to liver preparations completely prevented inhibition of cell growth. Liver preparations exhibit antiviral properties, which are probably due to lack of arginine. Most viruses (adeno, herpes, vaccina, reo, and papova) require arginine for their replication. (26 Refs)

* U. S. GOVERNMENT PRINTING OFFICE : 1982—361-847/114

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DNA TUMOR VIRUSES IN NON-PRIMATE SYSTEMS

SERIES CB11 No. 82/05 MAY, 1982

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VIRUS ASSOCIATED CELL TRANSFORMATION AND HOST ALTERATION

1. INHIBITION OF ADENOVIRUS ONCOGENICITY IN HAMSTERS BY ADENO-ASSOCIATED VIRUS DNA.

de la Maza LM, Carter BJ
Dept. Pathology, Univ. California at Irvine, Irvine, CA, 92717
JNCI; 67(6):1323-1326 1981

Adeno-associated virus (AAV) DNA partially inhibited in Syrian golden hamsters the formation of tumors by the human adenovirus (Ad) type 12. Furthermore, defective interfering AAV particles or their DNA also reduced tumorigenesis. Defective AAV particles contain aberrant genomes with extensive deletions of the internal AAV DNA sequences. Variant AAV DNA, containing 30% of the viral genome, decreased the incidence of Ad-induced tumors from 44 to 18%. Defective AAV particles, which have a buoyant density of 1.32 g/cm3 in CsCl and which are highly enriched for the DNA of the terminal regions (map positions, 0-5 and 95-100), completely suppressed Ad oncogenicity. This observation suggested that the AAV DNA sequence s close to the terminal region of the genome

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mediated the inhibition of the Ad oncogenicity. (Author abstract) (20 Refs)

2. DISTRIBUTION AND LIFE HISTORY OF RAT BRAIN TUMORS INDUCED BY HUMAN ADENOVIRUS TYPE 12 IN THEIR EARLY STAGES OF THE ONCOGENESIS.

Shinozaki Y
2nd Dept. Pathology, Okayama Univ. Medical Sch., 2-5-1, Shikata-cho, Okayama, Japan
Pathol Res Pract; 172(4):330-348 1981

Newborn rats were intracranially inoculated with human adenovirus Type 12 within 24 hr after birth and their brains were sequentially investigated by conventional histological techniques and autoradiography to clarify the early process of tumor growth. At the end of the 2nd wk atypical cells having bizarre configuration first appeared singly in the tapetum. From the end of the 3rd wk small clusters of slightly atypical subependymal cells ('abnormal clusters') appeared in the subependymal area. From the end of the 4th wk a few 'microtumors' showing compact nodular growth of atypical glial cells were observed among the 'abnormal clusters'. These early neoplastic changes in the rat brains had close relationship to subependymal cells. The perinatal subependymal cells showed most dense distribution in the subependymal area of lateral ventricle with open ventricular space ('open ventricular area') especially in the region of anterior horn. In contrast, the microtumors and the enlarged tumors were recognized much more in number in the subependymal area of bulbus olfactorius and tapetum where ventricular cavities closed in the young period of maturation ('closed ventricular area'). 'Abnormal clusters' were located in both of the areas with more preponderance in the open ventricular area. The reasons for this discrepancy of occurrence were discussed from the viewpoint of environmental factors. Subsequent investigation revealed that the av number of microtumors, after reaching its peak at the end of the 5th wk, diminished gradually and, finally, only one or two enlarged tumors were found per each tumor-bearing animal. The mechanisms for this phenomenon are open to further investigation. (Author abstract) (21 Refs)

3. TRANSFORMATION BY ADENOVIRUS EARLY REGION 2A TEMPERATURE-SENSITIVE MUTANTS AND THEIR REVERTANTS.

Logan J, Nicolas JC, Topp WC, Girard M, Shenk T, Levine AJ
Dept. Microbiology, State Univ. New York, Sch. Medicine, Stony Brook, NY, 11794
Virology; 115(2):419-422 1981

H5ts107 is a temperature-sensitive (ts) mutant of adenovirus Type 5 whose alteration maps in the structural gene for the viral DNA binding protein. Temperature-independent revertants of this mutant that form plaques at 39 C in HeLa cells have been isolated. These revertants fall into two classes: (1) ts+ revertants for growth and plaque formation at 39 C in both HeLa cells and 293 cells, a human cell line transformed by Type 5 adenovirus; (2) ts+ for growth and plaque formation at 39 C in HeLa cells, but ts for growth and plaque formation in 293 cells. The frequency of rat cell transformation by H5ts107 was fivefold higher than that of wild-type adenovirus. A Class 1 revertant, r(st107)127, transformed cells at the wild-type virus frequency while a Class 2 revertant, r(ts107)202, retained the ability to transform cells at the higher frequency. When the XhoI-C DNA fragment from these viruses, which contains the left end 15.5% of the viral genome, was employed to transform rat cells, then Ad5 wt, H5ts107, r(ts107)127, and r(ts107)202 DNAs all transformed with approximately equal frequencies. (Author abstract) (17 Refs)

4. INTEGRATION OF FOREIGN GENOME FRAGMENTS INTO CELLS TRANSFORMED OR COTRANSFORMED WITH FRAGMENTED ADENOVIRAL DNA.

Tikchonenko TI, Chaplygina NM, Kalinina TI, Gartel AL, Ponomareva TI, Naroditsky BS, Dreizin RS
D. I. Ivanovsky Inst. Virology, Acad. Medical Sciences USSR, Moscow 123098, USSR
Gene; 15(4):349-359 1981

DNA integration of highly oncogenic simian adenovirus type 7 (SA7) and non-oncogenic human adenovirus type 6 (Ad6) into the genome of newborn rat kidney cells transformed by fragmented DNA preparations was studied. SA7 and Ad6 transformation, expression of integrated viral sequences, Ad6-pBR322 plasmid cointegration comprised the study. Intact DNA transformation, transformation by fragmented DNA, shortening of integrated DNA, and competence of eukaryotic cells are discussed. Reassociation kinetics and spot hybridization were employed. DNA was fragmented with the specific endonucleases SalI and endonuclease BgIII. Cells transformation by fragmented DNA resulted in integration of the left-hand fragment and other regions of the viral genome with the oncogene. Integrated fragments were stable during numerous passages of cell lines. In the case of the Ad6-transformed cell line integrated fragments were not expressed. Mass losses of 25-50% occurred with the integration of SA7 DNA fragments. The linear form of pBR322 plasmid contributed to the Ad6 DNA integration into the genome of the transformed cell. (40 Refs)

5. VIRAL MESSENGER RNAS IN SIX LINES OF ADENOVIRUS-TRANSFORMED CELLS.

Lewis JB, Mathews MB
(c/o Mathews), Cold Spring Harbor Lab., P.O. Box 100, Cold Spring Harbor, NY, 11724
Virology; 115(2):345-360 1981

We have compared the proteins translated in vitro using virus-specific mRNA from six lines of adenovirus-transformed cells (F4, F17, F19, REM, T2C4, and 293) with those made using RNA from productively infected cells. The mRNAs for proteins E1A-58K, 54K, 48K, and 42K, and for E1B-15K, were detected in all cell lines and for E1B-57K in at least five of the lines. Neither of the minor E1A mRNAs (for proteins 25K and 28K) found under specific conditions in infected cells were found in any transfo rmed cell line. Two cell lines, F4 and T2C4, contained small amounts of other mRNAs encoded within region E1B including in the latter case one not detected in infected cells. The lines F4, REM, and T2C4 contain at least some E4 mRNAs, again including in the latter case one not detected in infected cells. T2C4 cells also contain E3 mRNAs, E2A-72K protein mRNA, and two E2B mRNAs, although the 32B proteins are not identical to any found in infected cells. The F19 and T2C4 lines express mRNAs encoded between 11 and 17 map units, the 'IVa2 region', but do not include the mRNA for polypeptide IVa2 itself. Some early mRNAs, including the 'immediate early' mRNAs, have not been detected in any of these lines of transformed cells: nor did the cell lines contain any mRNAs found exclusively at late times during productive infection. Comparison of these results with the properties of the cells tentatively suggest a correlation (although not necessarily causal) between the expression of additional viral genes and increased tumorigenicity. (Author abstract) (58 Refs)

6. DNA TOPOLOGICAL LINKING NUMBERS IN MALIGNANTLY TRANSFORMED SYRIAN HAMSTER CELLS.

Luchnik AN, Glaser VM
Laboratory of Biochemistry, Institute of Developmental Biology, Vavilov st. 26, Moscow 117334, USSR
MGG; 183(3):553-556 1981

The topological linking numbers in closed superhelical loops of nuclear DNA were measured as the density of DNA topological turns ('titratable superhelical turns') per unit length of DNA by means of sedimentation of superhelical DNA (in nucleoids) in gradients of 15-30% sucrose containing 1.95 M NaCl and various concentrations of ethidium bromide. In four malignantly transformed Syrian hamster cell lines (3 SV40-transformed and 1 spontaneous) the density of DNA topological turns was equal to or higher than the density of DNA topological turns in early passage embryonal Syrian hamster cells (absolute value of delta greater than 0.076) and, in contrast to normal cells, the malignant ones did not decrease the density of their DNA topological turns upon cultivation. it is proposed that the persistence of high densities of DNA topological turns in malignant cells is responsible for activation of transcription of a number of genes during malignant transformation. (Author abstract) (19 Refs)

7. INDUCTION OF CHROMOSOME ABERRATIONS IN MAMMALIAN CELLS BY TEMPERATURE-SENSITIVE SV40 MUTANT.

Gorbunova LV, Varshaver NB, Lelikova GP, Shapiro NI
Inst. Molecular Genetics, Moscow, USSR
Biol Nauki; (10):75-78 1981

To evaluate the mutagenic effect of simian virus SV40, cultured Syrian hamster cells were infected with temperature-sensitive (ts) SV40 mutant at 33 C and 40 C. Significant increase of the frequency of chromosome aberrations was recorded only at permissive temperature (33 C), while at restric tive temperatur (40 C), SV40-ts failed to induce chromosome aberrations. (13 Refs) (In RUS)

8. THE DISSECTION AND RECONSTRUCTION OF GENES AND CHROMOSOMES (NOBEL ADDRESS).

Berg P
Dept. Biochemistry, Stanford Univ. Sch. Medicine, Stanford, CA, 94305
Angew Chem; 93(10):885-893 1981

The development of understanding of the structure and function of genes and chromosomes is reviewed. Early research established that genes controlled the production of proteins. Analysis of DNA from viruses and bacteria disclosed the double-helix structure and purine-pyrimidine base-pair composition of DNA. Studies with polyoma and SV40 viruses allowed mapping of genes producing specific proteins. SV40 was shown to have different life cycles in primate and non-primate cells. Transformation of host cells by SV40 viruses was demonstrated, indicating that viruses could produce heritable changes in host-cell genetic material. Recombination experiments combined viral DNA from SV40 and bacterial DNA from Escherichia coli. Viral and bacterial DNA have since been incorporated into the genome of mammalian cells. Mammalian cells with bacterial genes have been shown to produce bacterial enzymes and to utilize metabolic pathways not used by normal cells. Findings of recombinant genetic studies have potential applications in medicine for correction of genetic defects and other problems. (49 Refs) (In GER)

9. ROLE OF ONCOGENES IN THE DEVELOPMENT OF TUMORS.

Klein G
Institutionen for tumorbiologi, Karolinska institutet, Stockholm, Sweden
Lakartidningen; 78(41):3575-3577 1981

Recent studies in viral oncogenes are reviewed. The coupling of some normal genes is a common feature of all tumor RNA-transforming viruses. Unlike the RNA viruses, the DNA viruses belong to several greatly different groups. Production of the so-called T antigen is a common feature of all DNA tumor viruses. This antigen occurs in all transformed cells. There are at least three virus types (papilloma virus, Epstein-Barr virus (EBV), and hepatitis B virus) contribute to the development of certain human tumors, but their contributions are less direct than in the case of infectious diseases. Only papilloma virus induces tumors directly, and most of them are benign, but papilloma virus may play a certain role in the genesis of some malignant epithelial tumors as well. Hepatitis B virus first causes liver cirrhosis, a precancerous state, the development of the tumor clone being the result of abnormal regeneration. (7 Refs) (In SWE)

10. RECENT DISCOVERIES ON CELLULAR PROTEINS IN RELATION TO VIRALLY AND CHEMICALLY INDUCED CARCINOGENESIS.

Jornvall H
Kemiska Institutionen 1, Karolinska Institutet, S-104 01 Stockholm, Sweden
Lakartidningen; 78(41):3585-3586 1981

Recent studies on the molecular mechanisms of carcinogenesis suggest the existence of a cellular protein ('53K') common to virally and chemically induced cell transformations. This type of protein binds some T-antigens from oncogenic DNA viruses, including large T-antigen from SV40 and Epstein-Barr virus-determined nuclear antigen. Transformation-related proteins from oncogenic RNA viruses are similar to normal cellular proteins. Several of these proteins have phosphorylating activity, and some of them have been found to be protein kinases. Associations of such proteins with the plasma membrane have also been shown. These different connections between various tumor-related proteins and normal cellular proteins suggest the importance of gene determination in transformations. (11 Refs) (In SWE)

VIRUS REPLICATION AND EXPRESSION

11. THE STRUCTURES OF THE SPLICED MRNAS ENCODING POLYOMA VIRUS EARLY REGION PROTEINS.

Treisman R, Cowie A, Favaloro J, Jat P, Kamen R
(c/o Kamen) Transcription Lab., Imperial Cancer Res. Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, England
J Mol Appl Genet; 1(2):83-92 1981

Polyoma (Py) virus early region messenger (m)RNAs synthesized during productive infection of mouse 3T6 cells were characterized at the nucleotide level. In the establishment of basic structures, nuclease S1-resistant hybrids formed between tsa 'shift-up' cytoplasmic polyadenylated RNA and Py DNA restriction fragments which span the entire early region were

analyzed by one- and two-dimensional agarose gel electrophoresis. The two splice donors and two splice acceptors were positioned more precisely by high resolution S1-gel mapping with terminally labeled DNA probes and polyacrylamide gels. The nucleotide sequences across the three splice joints were established by cloning and sequencing partial complementary (c)DNA copies of the mRNAs. The present data, in combination with previously described data on the polyadenylated 3'-end and detailed analysis of the capped 5' ends, complete the description of a family of differentially spliced mRNAs able to encode the known early region gene products, small-T, middle-T, and large-T proteins. (31 Refs)

12. SEQUENCES AT THE CAPPED 5'-ENDS OF POLYOMA VIRUS LATE REGION MRNAS: AN EXAMPLE OF EXTREME TERMINAL HETEROGENEITY.

Cowie A, Tyndall C, Kamen R

Transcription Lab., Imperial Cancer Res. Fund Lab., Lincoln's Inn Fields, London WC2A 3PX, England

Nucleic Acids Res; 9(23):6305-6322 1981

We have localized with respect to the genomic DNA sequence the capped 5'-termini of polyoma virus late region mRNAs. A minimum of fifteen different purine termini were found within a 94 base pair region (66.36 to 68.12 map units, nt 5075-5168) immediately preceding the sequence determining the late region mRNA leader repeat (1-3). The most common termini occur at nearly every possible purine within a 25 bp sequence proximal to the leader repeat unit. These do not bear the usual positional relationship to a sequence resembling the 'TATA' box consensus. Deletion mutants lacking minor cap sites and sequences upstream from the principal cap sites were viable. A deletion mutant lacking one of the principal cap sites formed small plaques, while a slightly larger deletion further impinging on the principal cap site region was non-viable. The principal cap sites, which we assume to be transcriptional initiation points, are included in a DNaseI hypersensitive region of polyoma virus chromatin. (Author abstract) (51 Refs)

13. A REGION OF THE POLYOMA VIRUS GENOME BETWEEN THE REPLICATION ORIGIN AND LATE PROTEIN CODING SEQUENCES IS REQUIRED IN CIS FOR BOTH EARLY GENE EXPRESSION AND VIRAL DNA REPLICATION.

Tyndall C, La Mantia G, Thacker CM, Favaloro J, Kamen R

Transcription Lab., Imperial Cancer Res. Fund Lab., Lincoln's Inn Fields, London WC2A 3PX, England

Nucleic Acids Res; 9(23):6231-6250 1981

Deletion mutants within the Py DNA region between the replication origin and the beginning of late protein coding sequences have been constructed and analyzed for viability, early gene expression and viral DNA replication. Assay of replicative competence was facilitated by the use of Py transformed mouse cells (COP lines) which express functional large T-protein but contain no free viral DNA. Viable mutants defined three new nonessential regions of the genome. Certain deletions spanning the PvuII site at nt 5130 (67.4 mu) were unable to express early genes and had a cis-acting defect in DNA replication. other mutants had intermediate phenotypes. Relevance of these results to eucaryotic 'enhancer' elements is discussed. (Author abstract) (58 Refs)

14. ULTRAVIOLET IRRADIATION INHIBITS ENCAPSIDATION OF SIMIAN VIRUS 40 CHROMATIN.

Roman A, Edenberg HJ

Dept. Microbiology and Immunology, Indiana Univ. Sch. Medicine, Indianapolis, IN, 46223

J Virol; 40(3):729-734 1981

During normal maturation the majority of pulse-labeled simian virus 40 DNA progresses from chromatin to previrions and virions within 5 hr. UV light inhibits this progression. In heavily irradiated culture (108 J m(-2)) most of the simian virus 40 DNA synthesized immediately before irradiation remains as chromatin for at least 5 hr. This inhibition of maturation seems to be a result of the inhibition of protein synthesis. The data suggest that the pool of proteins required for maturation is sufficient to convert one-third of the simian virus 40 DNA molecules labeled in a 10-min pulse (at 33 hr postinfection) from chromatin to previrions and virions and is exhausted within 1 hr. (Author abstract) (26 Refs)

15. IN VITRO TRANSCRIPTION OF ADENOVIRUS.

Fire A, Baker CC, Manley JL, Ziff EB, Sharp PA

Center Cancer Res., Massachusetts Inst. Technology, Cambridge, MA, 02139

J Virol; 40(3):703-719 1981

A series of recombinants of adenovirus DNA fragments and pBR322 was used to test the transcriptional activity of the nine known adenovirus promoters in a cell-free extract. Specific initiation was seen at all five early promoters as well as at the major late promoter and at the intermediate promoter for polypeptide IX. The system failed to recognize the two other adenovirus promoters, which were prominent in vivo only at intermediate and late stages in infection. Microheterogeneity of 5' termini at several adenovirus promoters, previously shown in vivo, was produced in the in vitro reaction and indeed appeared to result from heterogeneous initiation rather than 5' processing. To test for the presence of soluble factors involved in regulation of mRNA synthesis, the activity of extracts prepared from early and late stages of infection was compared on an assortment of viral promoter sites. Although mock and early extracts showed identical transcription patterns, extracts prepared from late stages gave 5-to 10-fold relative enhancement of the late and polypeptide IX promoters as compared with early promoters. (Author abstract) (43 Refs)

16. PROTEIN KINASES ASSOCIATED WITH THE ADENOVIRUS SINGLE-STRANDED DNA-BINDING PROTEIN.

Cajean-Feroldi C, Loeb J, Megnenni S, Girard M

Unites de Physiologie des Virus et de Biochimie des Regulations, Institut de Recherches Scientifiques sur le Cancer, Boite postale 8, F-94800 Villejuif, France

Eur J Biochem; 120(1):79-87 1981

Protein kinase activities copurifying with the 72,000-Mr DNA-binding protein of adenovirus on DNA-cellulose chromatography and gel filtration in acrylamide/agarose have been partially characterized and purified. One of these kinases was found to phosphorylate efficiently the viral DNA-binding protein in vitro and to be stimulated severalfold by the addition of histones, protamine, or polyamines. The kinase does not, however, phosphorylate histones, protamine, casein, or phosvitin. A second protein kinase was also recovered from single-stranded DNA-cellulose which is able to phosphorylate the 72,000-Mr DNA-binding protein, but which is inhibited by the addition of histones. Phosphorylation in vitro of the 72,000-Mr

DNA-binding protein from the ts125 mutants of adenovirus by the histone-stimulated protein kinase was found to be thermosensitive. (Author abstract) (52 Refs)

17. SYNTHESIS OF AN UNSPLICED CYTOPLASMIC MESSAGE BY AN ADENOVIRUS 5 DELETION MUTANT.

Carlock L, Jones NC
(c/o Jones) Dept. Biological Sciences, Purdue Univ., West Lafayette, IN, 47907
Nature; 294(5841):572-574 1981

A mutant of adenovirus 5 (dl502) which contains a deletion within early region E la and prevents splicing of the normal Ela transcripts is described. Mapping of cytoplasmic RNA isolated from wild-type and dl502-infected HeLa cells indicated that an unspliced Ela message is synthesized and accumulates in the cytoplasm of dl502-infected cells. Comparison of the Ela-generated autoradiograph bands from wild-type and dl502 RNA preparations showed that the unspliced Ela product from dl502 accumulates in the cytoplasm to 25-50% of the levels of wild-type Ela products. It is thought unlikely that the unspliced message could have resulted from nuclear leakage. Two phenotypic properties of dl502 indicated that a defective, partially functional, Ela gene product was being synthesized. Increased synthesis of dl502, which is defective for growth on HeLa cells, was attributed to the synthesis of a partially functional Ela gene product required for productive growth, by dl502. The second indicator was detection of transcripts from other regions of the genome, although seen at reduced levels. The results indicated that an unspliced region Ela transcript accumulates in the cytoplasm of dl502-infected cells; splicing and the removal of intron sequences are not necessary prerequisites to the production of stable, cytoplasmic RNA from this particular gene. (27 Refs)

18. PATHOGENESIS OF HERPES SIMPLEX VIRUS INFECTIONS IN GUINEA PIGS.

Scriba M, Tatzber F
Sandoz Forschungsinstitut, A - 1235 Vienna, Austria
Infect Immun; 34(3):655-661 1981

The pathogenesis of herpes simples virus Types 1 and 2 has been studied in guinea pigs after inoculation by various routes (sc and id infection in footpads and vaginal infection). Clinical observations as well as virus isolation studies are reported. Herpes simplex virus Type 2 infection by all three routes of inoculation led to acute primary and recurrent lesions. Virus persisted in the nervous system, particularly in sensory ganglia, and locally at the site of inoculation. Herpes simples virus Type 1 infection induced no or very mild primary symptoms. Recurrent lesions were only observed after id inoculation. Invasion of the nervous system and consequent establishment of latent ganglionic infection was less efficient than after herpes simplex virus Type 2 infection. Peripheral persistence was, however, equally common. (Author abstract) (22 Refs)

19. EPSTEIN-BARR VIRUS-INDUCED DEOXYNUCLEASE AND THE REUTILIZATION OF HOST-CELL DNA DEGRADATION PRODUCTS IN VIRAL DNA REPLICATION.

Feighny RJ, Henry BE, Pagano JS
Cancer Res. Center, Univ. North Carolina, Sch. Medicine, Chapel Hill, NC, 27514
Virology; 115(2):395-400 1981

Extracts of Raji cells superinfected with EBV obtained from P3HR-1 producer cells revealed the presence of a DNase activity. Template specificity determinations disclosed exonuclease activity on both single-stranded and double-stranded molecules. The activity could be abolished by EBV antiserum. The DNase activity was detectable 18 hr after superinfection. A possible function for the enzyme was suggested by experiments in which radiolabel, which was initially present only at the density of cellular DNA, was chased into DNA banding at the density of EBV DNA. The chase of incorporated label was first observed 36 hr after superinfection and accounted for 10% of the total label present in both DNA peaks. The gradual shift of label reached a maximum of 20% of the total label by 72 hr after infection. Rate-velocity centrifugation analyses revealed that cellular DNA was degraded after superinfection. The use of DNA synthesis inhibitors suggested that the host-cell DNA degradation products were recycled into nascent EBV DNA via the cellular salvage pathway. (Author abstract) (15 Refs)

20. EPSTEIN-BARR VIRUS-INDUCED EARLY POLYPEPTIDES IN RAJI AND NC37 CELLS ACTIVATED BY DITERPENE ESTER TPA IN COMBINATION WITH N-BUTYRATE.

Kawanishi M, Sugawara K, Ito Y
(c/o Ito) Dept. Microbiology, Kyoto Univ., Kyoto 606, Japan
Virology; 115(2):406-409 1981

Epstein-Barr virus (EBV)-induced early polypeptides in activated nonproducer Raji and NC37 cells, and such polypeptides induced in producer P3HR-1 cells were studied for comparison. The major difference was that the two polypeptides with molecular weights of 140,000 (140K) and 120,000 (120K) which are presumably involved in viral DNA synthesis in the activated producer cells, were not detectable in the activated nonproducer cells. (Author abstract) (20 Refs)

VIRAL GENETICS

21. CHARACTERIZATION OF COLIPHAGE LAMBDA HYBRIDS CARRYING DNA FRAGMENTS FROM HERPES SIMPLEX VIRUS TYPE 1 DEFECTIVE INTERFERING PARTICLES.

Denniston KJ, Madden MJ, Enquist LW, Vande Woude G
Div. Molecular Virology, Georgetown Univ. Medical Sch., Rockville, MD, 20852
Gene; 15(4):365-378 1981

Characterization of 34 hybrid lambda bacteriophages carrying Eco RI fragments obtained from DNA of defective interfering particles of the Patton strain of Herpes simplex virus type 1 (HSV-1) is described. Recombinant DNA technology was used to study the structure of individual molecules within the population. Three structural groups of Patton defective DNA (dDNA) Eco RI fragments were defined. All cloned fragments contained S region terminal repeat sequences (TRS) fused to unique HSV-1 DNA. Several fragments contained deletions and rearrangements not described previously for DNA of HSV-1 defective interfering particles. A model describing the generation of defective interfering DNA based on recombination events involving the terminal 'a' sequence is presented. (32 Refs)

IMMUNOLOGICAL STUDIES

22. INHIBITION OF T CELL-MEDIATED FUNCTIONS BY MVM(I), A PARVOVIRUS CLOSELY RELATED TO MINUTE VIRUS OF MICE.

Engers HD, Louis JA, Zubler RH, Hirt B
Dept. Immunology and Virology, Swiss Inst. Experimental Cancer Res., 1066 Epalinges, Switzerland
J Immunol; 127(6):2280-2285 1981

A purified preparation of MVM(i), a murine parvovirus closely related to minute virus of mice (MVM), was found to inhibit various functions mediated by murine T cells in vitro. Addition of MVM(i) virus to secondary allogeneic mixed leukocyte cultures resulted in the inhibition of both lymphocyte proliferation (3H-thymidine incorporation) and the generation of cytolytic T lymphocyte activity but not interferon production. MVM(i) virus also inhibited the growth and cytolytic activity of several cloned, long-term Lyt-2+ cytolytic T cell lines. Furthermore, the antigen-induced proliferative responses of parasite- (Leishmania) specific Lyt-1+ T cells in vitro was abrogated by the addition of MVM(i) virus to the culture. Finally, the suppression of an in vitro antibody response to SRBC by MVM(i) virus was the result of the inhibition of T helper cells required for the B cell response. These suppressive effects were specific for MVM(i); parallel studies in which the prototype MVM parvovirus was used showed no significant inhibition in the various systems tested. (Author abstract) (32 Refs)

23. CELL-MEDIATED IMMUNITY TO HERPES SIMPLEX VIRUS: RECOGNITION OF TYPE-SPECIFIC AND TYPE-COMMON SURFACE ANTIGENS BY CYTOTOXIC T CELL POPULATIONS.

Eberle R, Russell RG, Rouse BT
Dept. Microbiology, Coll. Liberal Arts, Univ. Tennessee, Knoxville, TN, 37916
Infect Immun; 34(3):795-803 1981

In this communication, we examine the specificity of anti-herpes simplex virus (HSV) cytotoxic T lymphocytes (CTL). Serological studies of the two related HSV serotypes (HSV-1 and HSV-2) have revealed both type-specific and cross-reactive antigenic determinants in the viral envelope and on the surface of infected cells. by analysis of cytotoxicity of CTL generated in vitro by restimulation of splenocytes from mice primed with one or the other HSV serotype, the recognition of both type-specific and cross-reactive determinants on infected target cells by anti-HSV CTL was detectable. Thus, effector cells generated by priming and restimulating with the same virus recognized both type-specific and cross-reactive determinants on target cells infected with the homologous virus, but only cross-reactive determinants on target cells infected with the heterologous HSV serotype. CTL generated by restimulation with the heterologous virus were capable of recognizing only the cross-reactive determinants on either HSV-1-or HSV-2-infected target cells. These results indicate that two subpopulations of CTL exist in a population of anti-HSV immune spleen cells - those which recognize type-specific determinants and those specific for cross-reactive antigenic determinants present on the surface of HSV infected cells. The type-specific subset of anti-HSV CTL was shown to recognize the gC glycoprotein of HSV-1 infected target cells. In addition to the gC glycoprotein, at least one other type-specific surface antigen was also recognized by anti-HSV CTL in addition to the cross-reactive determinants recognized by anti-HSV CTL. (Author abstract) (23 Refs)

OTHER RELATED STUDIES

24. A SMALL SEGMENT OF POLYOMA VIRUS DNA ENHANCES THE EXPRESSION OF A CLONED BETA-GLOBIN GENE OVER A DISTANCE OF 1400 BASE PAIRS.

de Villiers J, Schaffner W
Institut fur Molekularbiologie II, Universitat Zurich, Honggerberg 8093, Zurich, Switzerland
Nucleic Acids Res; 9(23):6251-6264 1981

The hemoglobin beta-1 gene of the rabbit was linked to a 244 bp DNA fragment from the beginning of the polyoma virus late region, not including the viral origin of replication. After transfection of such recombinant DNAs into mouse 3T6 and human HeLa cells, the polyoma sequences were found to strongly enhance the level of correct beta-globin gene transcripts over a distance of at least 1400 bp. These findings are similar to those obtained with a segment of DNA from the corresponding region of the SV40 genome which, however, shows very limited sequence homology to the polyoma 244 bp segment. Using the same assay, a complete copy of polyoma virus DNA was found to interfere with the enhancement of globin gene expression in a cell type-specific manner which may be due to incorrect transcription. In contrast to the complete polyoma virus genome, the 244 bp DNA fragment will be particularly useful as a component of mammalian expression vectors since it almost exclusively yielded high levels of correct beta-globin gene transcripts. (Author abstract) (40 Refs)

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VIRUS ISOLATION/ STRUCTURE/ CHARACTERIZATION/ IDENTIFICATION

1. THREE SEGMENTS FROM THE MONKEY GENOME THAT HYBRIDIZE TO SIMIAN VIRUS 40 HAVE COMMON STRUCTURAL ELEMENTS.

Queen C, Lord ST, McCutchan TF, Singer MF
Lab. Biochemistry, NCI, Bethesda, MD, 20205
Mol Cell Biol; 1(12):1061-1068 1981

Recently, three cloned segments were isolated from a monkey genomic library. The segments hybridizing to the HindIII-C fragment of the DNA virus, simian virus 40 (SV40) are discussed. The primary structure of one segment has been reported. As a further step in analyzing the relationship between the SV40-hybridizing segments and SV40 itself, the sequence and genomic arrangements of the other two cloned segments was determined by previously described methods, and a comparison was made of all three. The SV40 hybridizing region in each segment was limited to several hundred base pairs. All segments contained multiple and disconnected sequences homologous to the region of SV40 directly surrounding the viral replication origin. While the number and arrangement of the

HE 20.3173/2:

homologous sequences was different in the three segments, each had the following features in common: (1) each contained multiple copies of the sequence GGGCGGPuPu, which also appeared 6x near the origin of SV40, (2) each contained several strong homologies to the central dyad symmetry of SV40, and (3) each contained a long internal repeat, as did the origin region of SV40. These three SV40 hybridizing segments are members of a larger family of genomic sequences that hybridize well to each, but not necessarily to SV40. (18 Refs)

2. POLYOMA VIRUS CAPSID STRUCTURE AT 22.5 A RESOLUTION.

Rayment I, Baker TS, Caspar DL, Murakami WT

Rosenstiel Basic Medical Sciences Res. Center, Brandeis Univ., Waltham, MA, 02254

Nature; 295(5845):110-115 1982

X-ray diffraction data from polyoma capsid crystals were phased by refinement of low-resolution starting models to obtain a self-consistent structural solution. The unexpected result that the hexavalent morphological unit is a pentamer shows that specificity of bonding is not conserved among the protein subunits in the icosahedrally symmetric capsid. (Author abstract) (21 Refs)

3. CHARACTERISTICS OF BK PAPOVAVIRUS DNA PREPARED DIRECTLY FROM HUMAN URINE.

Mew RT, Lecatsas G, Prozesky OW, Harley EH

(c/o Harley) Dept. Chemical Pathology, Univ. Cape Town Medical Sch., Observatory 7925, South Africa

Intervirology; 16(1):14-19 1981

DNA was prepared directly from preparations of papovavirus excreted in the urine of two patients on immunosuppressive therapy, without preliminary amplification in cell cultures. EcoRI, BamHI, and HindIII restriction patterns were typical of BK virus DNA and were identical for the two isolates. However, there were significant differences in the sizes of the HindIII fragments when compared with those of standard cell-culture-adapted BK virus strains. (Author abstract) (13 Refs)

4. MULTIMERIC FORMS OF HERPES SIMPLEX VIRUS TYPE 2 GLYCOPROTEINS.

Eberle R, Courtney RJ

(c/o Courtney) Dept. Microbiology, Univ. Tennessee, Knoxville, TN, 37916

J Virol; 41(1):348-351 1982

Herpes simplex virus type 2-specific glycoproteins present in detergent extracts of infected cells were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under conditions designed to permit detection of multimeric forms of these glycoproteins. Two high-mol-wt glycosylated species were detected when samples were disrupted at lower temperatures or in the absence of any reducing agents. One multimer having an apparent mol wt of 275,000 was identified as a multimer of the gA or gB glycoprotein or both. The second glycoprotein, having a mol wt of approx 230,000, was identified as a multimeric form of the gC glycoprotein. These data indicate that the gC as well as the gA and gB glycoproteins of herpes simplex virus type 2 may exist in a multimeric form. (Author abstract) (13 Refs)

5. VIRUSES SIMILAR TO HEPATITIS B VIRUS (ICRONS).

Blumberg BS

Inst. Cancer Res., Fox Chase Cancer Center, Philadelphia, PA, 19111

Hum Pathol; 12(12):1107-1113 1981

In 1971, on the basis of the unusual clinical and epidemiologic characteristics of hepatitis B virus, we postulated the existence of a class of viruses that we termed icrons. An increased understanding of the molecular biology of hepatitis B virus resulted in the discovery, by Summers and his colleagues, of the woodchuck hepatitis virus. This virus is common in the North American woodchuck (Marmota monax) and is associated with primary cancer of the liver in this animal. Subsequently similar viruses were found in Beechey ground squirrels in California by Marion and her coworkers and domesticated (Pekin) ducks from the United States by Mason and his coworkers. In the latter the virus infects the liver and presumably is associated with disease of this organ. The discovery of additional viruses similar to hepatitis B virus in animals other than man and their association with cancer of the liver encourages the continuing search for other virus-cancer associations for which prevention methods might be effective. (Author abstract) (20 Refs)

6. SIMILARITIES AND DIFFERENCES IN THE FC-BINDING GLYCOPROTEIN (GE) OF HERPES SIMPLEX VIRUS TYPES 1 AND 2 AND TENTATIVE MAPPING OF THE VIRAL GENE FOR THIS GLYCOPROTEIN.

Para MF, Goldstein L, Spear PG

Dept. Medicine, Ohio State Univ. Coll. Medicine, Columbus, OH, 43210

J Virol; 41(1):137-144 1982

We performed affinity chromatography and immunoprecipitation experiments to determine whether cells infected with herpes simplex virus type 2 (HSV-2) expressed a glycoprotein that was functionally and antigenically related to the HSV-1 Fc-binding glycoprotein designated gE. We found that a protein from extracts of HSV-2-infected HEp-2 cells bound specifically to an Fc affinity column and that the electrophoretic mobility of this protein in sodium dodecyl sulfate-acrylamide gels was slightly less than the mobility of HSV-1 gE. Immunoprecipitation experiments performed with an antiserum prepared against HSV-1 gE revealed that (i) extracts from HSV-2-infected cells contained a glycoprotein that was antigenically related to HSV-1 gE; (ii) the electrophoretic mobility of the HSV-2 gE was indistinguishable from the mobility of the HSV-2 Fc-binding protein; (iii) the antiserum reacted with both newly synthesized transient forms and stable fully processed forms of both HSV-1 gE and HSV-2 gE; and (iv) the transient and stable forms of HSV-2 gE all had lower electrophoretic mobilities than their HSV-1 counterparts. Electrophoretic analyses of gE precipitated from extracts of HEp-2 cells infected with two sets of HSV-1 x HSV-2 intertypic recombinant viruses suggested that the gene for gE is located at the right end of the HSV genome (0.85 to 0.97 map units) in the unique portion of the S component. (Author abstract) (20 Refs)

7. CLONING OVERLAPPING DNA FRAGMENTS FROM THE B95-8 STRAIN OF EPSTEIN-BARR VIRUS REVEALS A SITE OF HOMOLOGY TO THE INTERNAL REPETITION.

Buell GN, Reisman D, Kintner C, Crouse G, Sugden B
McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706
J Virol; 40(3):977-982 1981

Overlapping, sheared DNA fragments from the B95-8 strain of Epstein-Barr virus were cloned in Charon 4A. Eleven recombinant phages plus one recombinant plasmid contained all of the sequences found in B95-8 virion DNA. Analysis of recombinant DNA molecules revealed a previously undetected site of homology to the internal repetition found in Epstein-Barr virus DNA. This site was adjacent to or at a site which was unstable when the recombinant DNA was propagated as phage DNA in prokaryotic hosts. (Author abstract) (14 Refs)

VIRUS ASSOCIATED CELL TRANSFORMATION AND HOST ALTERATION

8. ALTERED PROPERTIES OF TUMORS INDUCED BY ADENOVIRUS TYPE 12 DNA FRAGMENT TRANSFORMED CELLS AFTER GROWTH IN IMMUNOCOMPETENT RATS.

Moriuchi T, Yamashita T, Imamura M, Fujinaga K
Dept. Molecular Biology, Cancer Res. Inst., Sapporo Medical Coll., S-1, W-17, Chuo-ku, Sapporo 060, Japan
Int J Cancer; 29(1):101-105 1982

The GY1-3-1 cell line, which was derived from a rat cell line transformed by the Ad 12 HindIII-G fragment, can induce tumors in newborn rats, but not in mature rats. However, when 10 mature rats were transplanted with a large number of GY1-3-1 tumor fragments, two developed tumors with a long latency period. These two tumors (GY1-3M and GY1-3Y) were quite different in properties from the originally inoculated GY1-3-1 tumor. Tumor transplantation studies revealed that both GY1-3M and GY1-3Y tumors could grow in mature rats, and the number of takes with transplanted tumor was 100% with GY1-3M tumor and 40% with GY1-3Y tumor. Histopathologically original GY1-3-1 tumor showed a morphology of undifferentiated sarcoma, while the histologic picture of GY1-3M and GY1-3Y tumors was similar to that encountered in human fibrosarcoma and malignant fibrous histiocytoma, respectively. Both GY1-3M and GY1-3-1 tumors, when recultured in vitro, gave rise to cell lines with a fibroblastic appearance, although the original GY1-3-1 cell line exhibited an epithelioid morphology. In chromosome analysis, the GY1-3-1 cell line was pseudodiploid, while the cell lines from GY1-3M and GY1-3Y tumors were hyperdiploid or near-triploid. The metacentri c marker chromosome, M1, was present in mitotic cells of the GY1-3-1 line and of the GY1-3M tumor lines, but absent from the GY1-3Y tumor lines. By Southern blot hybridization, multiple bands of cellular DNAs from parental GY1-3-1 cells hybridized with labeled Ad12 HindIII-G, while only a single band hybridized in DNAs from both GY1-3M and GY1-3Y tumors. (Author abstract) (15 Refs)

9. RESTRICTED REPLICATION OF MOUSE ADENOVIRUS STRAIN FL IN MOUSE BALB/C CELLS TRANSFORMED BY SIMIAN ADENOVIRUS 7.

Dussaix E, Faucon-Biguet N, Samolyk D, Boni C, Tournier P
Service de Microbiologie, Hopital de Bicetre, 94270 Le Kremlin-Bicetre, France
Virology; 116(2):641-645 1982

Mouse kidney cells (Balb/c cells), in which mouse adenovirus strain FL (Ad FL) replicates, become restricted in their capacity to support Ad FL growth after transformation by simian adenovirus 7 (SA7). In transformed cells (Balb/c SA7 cells), the duration of the Ad FL cycle is significantly longer than in normal cells; some virus DNA replication and virus protein synthesis occur but the yield of infectious Ad FL particles is greatly reduced. Only a few cells produce virus as estimated by the number of infectious centers. However, no significant differences are noted in the adsorption and in the penetration of Ad FL in both cell types. Thus, the restrictive event in virus multiplication in Balb/c SA7 cells occurs probably after viral uncoating and before viral DNA synthesis. (Author abstract) (14 Refs)

10. DNA MEDIATED GENE TRANSFER USING SIMIAN VIRUS 40 OR POLYOMA VIRUS MORPHOLOGICAL TRANSFORMATION AS SELECTIVE MARKERS: BRIEF REPORT.

Zouzias D, Bertelsen AH, Rush MG
Dept. Pathology, New York Univ. Sch. Medicine, 550 First Ave., New York, NY, 10016
Arch Virol; 70(3):291-295 1981

The stable uptake of a bacterial plasmid by mouse or rat cells has been detected following the respective co-transfection of these cells with plasmid in the presence of either Simian virus 40 (SV40) or Polyoma virus DNAs. About 60-70% of the resulting SV40-transformed mouse cell clones and about 40% of the Polyoma transformed rat cell clones contained plasmid DNA sequences. (Author abstract) (10 Refs)

11. LATE MESSENGER RNA PRODUCTION BY VIABLE SIMIAN VIRUS 40 MUTANTS WITH DELETIONS IN THE LEADER REGION.

Piatak M, Subramanian KN, Roy P, Weissman SM
Dept. Human Genetics, Yale Univ. Sch. Medicine, New Haven, CT, 06510
J Mol Biol; 153(3):589-618 1981

We have analyzed the structure of simian virus 40 messenger RNA in cells infected with mutant viruses in which DNA encoding various segments of the late leader has been deleted. The results show that large amounts of stable unspliced mRNA may accumulate in the cytoplasm and that some unspliced RNA appears in polyribosomes. The locations of the prominent 5' ends of mRNA are influenced by relatively remote downstream sequences and bear no fixed relationship to any sequence of the simian virus 40 DNA retained in the mutant virus. Sequences downstream from the leader template may play a role in determining the region in which transcription initiates preferentially. Splices may occur even when nucleotides further than seven residues downstream from the upstream end of the splice are altered, and alternative splices with one junction in common may be differently affected by deletions near the common splice junction. (Author abstract) (64 Refs)

12. T ANTIGEN REPRESSION OF SV40 EARLY TRANSCRIPTION FROM TWO PROMOTERS.

Hansen U, Tenen DG, Livingston DM, Sharp PA
Center for Cancer Res., Massachusetts Inst. Technology, Cambridge, MA, 02139
Cell; 27(3):603-612 1981

The SV40 early mRNAs encode large (T) and small (t) tumor antigens. During the lytic cycle, the 5' termini of the early mRNAs undergo a shift: shortly after infection only an initiation site downstream from the TATA box is utilized; later an upstream initiation site becomes prominent. Both initiation sites are utilized in an in vitro transcription extract. D2T, a T antigen analog, specifically represses transcription in vitro from both initiation sites, but at different concentrations. Binding of D2T to Site I suppresses initiation from the site downstream of the TATA box; binding to Sites II and I suppresses initiation from the upstream site. The role of T antigen binding in repression of transcription and in the shift of initiation sites on the early strand is discussed. (Author abstract) (52 Refs)

13. REVERSION FROM DEFICIENCY OF GALACTOSE-1-PHOSPHATE URIDYLYLTRANSFERASE (GALT) IN AN SV40-TRANSFORMED HUMAN FIBROBLAST LINE.

Benn PA, Kelley RI, Mellman WJ, Amer L, Boshes FS, Markus HB, Nichols W, Hoffman B
Prenatal Diagnosis Lab. New York City, 455 First Ave., New York, NY, 10016
Somatic Cell Genet; 7(6):667-682 1981

Control SV40-transformed human fibroblasts can be readily adapted to growth on medium containing galactose as sole hexose source (galactose-MEM). Most cells from a line of SV40-transformed skin fibroblasts from a patient with galactose-1-phosphate uridylyltransferase (GALT) deficiency died in galactose-MEM. Two GALT revertant clones were studied and the usefulness of SV40-transformed cell lines for studying human mutation at a well-characterized genetic locus was demonstrated. The expression of GALT in two lines was stable in nonselective conditions. Reaction max velocities were different in each line. Velocities were compared to residual activity in the parental cell strain. Both lines demonstrated heat-inactivation profiles for GALT that differed from the control. Uridine diphosphoglucose concentration altered significantly the thermostability of GALT. The GALT protein had amounts comparable to that of the parental cell strain. The electrophoretic mobility of GALT differed from control cells. The structural gene changes were probably responsible for the apparent reversion in the studied two lines. The ability to obtain a number of different GALT revertants enables to study the GALT defects at a molecular level. (25 Refs)

14. INTERACTION OF SV40 T-ANTIGEN WITH CHROMATIN IN TUMOR CELLS.

Rheszowska-Wolny I, Sadzinska L, Grobner S, Shliankevich MA, Drize OB, Demina EB, Polotskaia AV
Inst. Oncology, Gliwice, Poland
Biokhimiia; 46(10):1880-1886 1981

Sedimentation analysis and electrophoresis in the polyacrylamide gel showed the presence of T-antigen of simian virus SV40 in the chromatin isolated from SV40-induced Syrian hamster tumors. T-antigen was bound to oligonucleosomes fraction. It was suggested that T-antigen interacts not only with DNA but also with H1 histone. (24 Refs) (In RUS)

15. FOLATE POLYGLUTAMATE AND MONOGLUTAMATE ACCUMULATION IN NORMAL AND SV40-TRANSFORMED HUMAN FIBROBLASTS.

Hoffman RM, Coalson DW, Jacobsen SJ, Erbe RW
Dept. Pediatrics, M009, Univ. California, San Diego, La Jolla, CA, 92093
J Cell Physiol; 109(3):497-505 1981

Folate poly- (PG) and monoglutamate (MG) accumulation was determined in normal diploid and SV40-transformed human fibroblasts. After cells were depleted of folates, limiting amounts of 3H-folic acid were provided, which resulted in similar growth rates for both cells. Both normal and transformed cells accumulated PG and MG, but by 72 hr, transformed cells contained 3-10x more PG than normal cells. Thus, inhibition of polyglutamate formation may have potential as a mechanism in chemotherapy. (21 Refs)

16. POLYOMA T ANTIGENS.

Eckhart W
Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
Adv Cancer Res; 35:1-25 1981

Current knowledge on polyoma T proteins is reviewed, including the role of these viral-coded proteins in lytic infection of mouse cells, DNA nucleotide sequences (coding and regulatory signals), mutations affecting the T antigens, and cell transformation and tumorigenicity. The T antigens are compared structurally with SV40 in terms of nucleotide sequences. The roles of the large, small, and medium T antigens in lytic infection and transformation are discussed. The functions associated with small and medium T antigens, particularly attempts to detect protein kinase activity associated with polyoma T, are reviewed. (110 Refs)

17. SYNTHESIS AND STABILITIES OF PROTEINS RELATED TO THE POLYOMA SMALL T ANTIGEN IN ESCHERICHIA COLI.

Horwich A, Koop AH, Eckhart W
Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
Mol Cell Biol; 2(1):88-92 1982

Early proteins (T antigens) encoded by polyoma and simian virus 40 which are involved in virus replication and neoplastic cell transformation were found to also be synthesized in Escherichia coli. A protein with N terminus derived from polyoma was significantly more unstable than a similar protein with N terminus derived from beta-galactosaminidase. (6 Refs)

18. INTEGRATION SITE OF POLYOMA VIRUS DNA IN THE INDUCIBLE LPT LINE OF POLYOMA-TRANSFORMED RAT CELLS.

Mendelsohn E, Baran N, Neer A, Manor H
Dept. Biology, Technion-Israel Inst. Technology, Haifa, Israel
J Virol; 41(1):192-209 1982

The structure of polyoma virus (Py) integration site in the inducible LPT line of Py-transformed rat cells was determined by biochemical methods of gene mapping. The data indicate that LPT cultures contain Py insertions of various lengths integrated into the same chromosomal site in all the cells. The length heterogeneity of the viral insertions is due to the presence of 0, 1, 2, or 3 Py genomes arranged in a direct tandem repeat within invariable sequences of viral DNA. Double-digestion experiments were also carried out with the above enzymes and with enzymes that cleave the Py genome at multi-

ple sites. The data obtained in these experiments were used to construct a physical map of the integration site. This map showed that the early region of the virus remained intact even in the smallest insertion (which contains no whole duplicated genomes), whereas the late region was partially duplicated and split during integration. The smallest insertion is colinear with the Py physical map over a region including the entire Py genome and at least a part of the duplicated segment. This structure could give rise to nondefective circular viral DNA molecules by single homologous recombination events. Similar recombination events may occur at a higher frequency in the longer insertions, which include longer regions of homology, and may yield many more free viral genomes. The presence of these insertions in LPT cells could thus by one of the factors which account for the high inducibility of the LPT line. (34 Refs)

19. THE EVOLUTION OF POLYOMA-TRANSFORMED RAT CELL LINES DURING PROPAGATION IN VITRO.

Dailey L, Colantuoni V, Fenton RG, La Bella F, Zouzias D, Gattoni S, Basilico C

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Virology; 116(1):207-220 1982

We have previously shown that the presence of a functional viral large T antigen in polyoma-transformed rat cells results in instability of the integrated viral DNA, as manifest by free viral DNA production, excision, and amplification of the integrated viral molecules. It has also been observed that polyoma tumors or transformed cell lines often do not produce functional large T antigen. To determine if an evolution toward the loss of large T-Ag function took place in vitro in polyoma-transformed cell lines, we studied the changes occurring in the integrated viral DNA sequences of a ts-a Py-transformed rat cell line, ts-a H3, upon propagation under conditions permissive (33 C) or nonpermissive (39 C) for large T function. H3 contains a single insertion of a partial tandem of approx 1.2 viral genomes, and at 33 C produces free viral DNA and also undergoes a high frequency of excision and amplification, involving recombination between homologous portions of viral DNA. While the integrated arrangement of viral DNA sequences remains unchanged at 39 C, in two independent experiments long-term propagation of H3 at 33 C resulted in the emergence of a fully transformed cell population which no longer produced free viral DNA and whose integrated viral DNA pattern was modified. In one of these populations, H3, interruption of the integrated viral coding sequences unique to large T antigen by a segment of host DNA resulted in the loss of large T antigen function. In another population, H3A*-3, the integrated viral DNA had undergone some rearrangement which, however, did not apparently affect large T Ag coding sequences. Since the latter cell population did not produce free viral DNA and was unable to 'rescue' integrated viral DNA sequences from A t-Ag-negative Py-transformed cell line after cell fusion, the large antigen produced by H3A*-3 is probably nonfunctional. These results are consistent with the hypothesis that the presence of large T antigen leads to instability of the viral DNA integration and that this instability is responsible for the selection of large T antigen-negative cells from Py-transformed populations. Possible mechanisms underlying this type of evolution are presented. (Author abstract) (33 Refs)

20. TRANSFORMATION OF HAMSTER KIDNEY CELLS BY FRAGMENTS OF BK VIRUS DNA.

Grossi MP, Corallini A, Valieri A, Balboni PG, Poli F, Cavuto A, Milanesi G, Barbanti-Brodano G

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J Virol; 41(1):319-325 1982

Hamster kidney cells were transformed, with comparable efficiency, by circular or linear molecules of complete BK virus (BKV) genome and by agarose gel-purified fragments of BKV DNA obtained by single or double digestions with various restriction endonucleases. Only fragments containing the complete early region of BKV DNA displayed transforming activity. Analysis by blot hybridization of the arrangement of viral DNA sequences in a cloned cell line transformed by a 3.8-kilobase fragment, obtained after sequential digestion of BKV DNA with HhaI and BamHI, showed the presence of seven viral integrations into the cellular DNA. Apparently all of the integrated viral molecules contained the entire early region of BKV DNA. Large T antigen, small t antigen, and the 56,000-dalton nonviral Tau antigen were detected in transformed cells by immunoprecipitation. The pattern of integration of viral sequences in transforme d cells was constant over many generations. Likewise, large T antigen was always detected in transformed cells at various passage levels. These results may suggest that all of the sequences of the early region coding for large T antigen are required for transformation by BKV. Alternatively, subgenomic segments of the BKV DNA early region may be unable to transform because the appropriate polyadenylation site, necessary to obtain a complete functional transcriptional unit, is removed by the restriction enzyme cleavage. (Author abstract) (34 Refs)

21. HIGH FREQUENCY OF GENE TRANSFER AFTER FUSION BETWEEN BACTERIA AND EUKARYOTIC CELLS.

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Nature; 295(5846):257-259 1982

The possibility of producing stable transformants using eukaryotic simian virus 40 (SV40) genes carried in a bacterial plasmid was studied. SV40 genes can be directly transferred into mammalian cells in culture; after polyethylene glycol-induced fusion with bacterial protoplasts, a fraction of the recipient cells yields infectious virus. Cells from Escherichia coli strain 1106 carrying one of the plasmids were grown to a density of 2 x 10(8) cells/ml. Transformed foci were observed after fusion of FR3T3 rat cells with bacteria carrying plasmid pPY-1. Efficiency of focus formation after transfer of either polyoma virus or SV40 early genes was at least equal to focus transformation observed after infection with virions. At high ratios of bacteria/recipient cells, all the cells expressed the early viral proteins 48 hr after fusion. Under optimal conditions, transfer by fusion appeared to be 10-to 20-fold more efficient than DNA transfection by the Ca++ co-precipitation technique for introduction of foreign genes into eukaryotic recipients. A systematic analysis of the parameters involved indicated that the extent of lysosome treatment was critical. The chloramphenicol amplification step seems to be essential; in several cases where the step was omitted, using either pBR322 or pAT153 plasmid, no transformants were produced after fusion. Another important parameter that must be considered is the choice of bacterial strain. (12 Refs)

22. TRANSFORMATION INDUCED BY HERPES SIMPLEX VIRUS: A POTENTIALLY NOVEL TYPE OF VIRUS-CELL INTERACTION.

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Lab. Molecular Virology, NCI, Frederick, MD, 21701
Adv Cancer Res; 35:27-47 1981

Three areas of the literature on herpes simplex virus (HSV) transformation induction are reviewed: HSV-induced transformation in vitro, the contrast between HSV in vitro transformation studies and transformation induced by other DNA viruses, and mechanisms of transformation in the HSV system. Models for in vitro transformation by HSV include use of nonpermissive cells, use of virus- host range mutants, use of inactivated viruses, use of temperature-sensitive virus mutants, and use of virus DNA or restriction enzyme-digested DNA fragments. Properties of biochemically transformed cells, morphologically, and tumorigenically transformed cells, and persistence of HSV genetic material in these cells, are discussed. The major problem remaining in elucidating the role of HSV in transformation is the lack of a system in which significant levels of transformation can be obtained reproducibly. (96 Refs)

23. EXPRESSION OF EPSTEIN-BARR VIRAL EARLY ANTIGEN IN MONOLAYER TISSUE CULTURES AFTER TRANSFECTION WITH VIRAL DNA AND DNA FRAGMENTS.

Grogan E, Miller G, Henle W, Rabson M, Shedd D, Niederman JC
Dept. Pediatrics, Yale Univ. Sch. Medicine, New Haven, CT, 06510
J Virol; 40(3):861-869 1981

Antigens associated with the Epstein-Barr virus (EBV) replicative cycle were found in the nucleus and cytoplasm of human placental, Vero, BSC-1, and owl monkey kidney cells transfected with EBV DNA prepared from several different strains of virus. The number of antigen-positive nuclei increased when transfection was followed by cell fusion induced by inactivated Sendai virus. About 1,200 antigen-positive foci were induced per ug of EBV DNA. On the basis of their reactivity with various well-characterized human sera, it appears that the antigens are part of the early antigen complex. None of the four restrictive endonucleases, EcoRI, HindIII, SalI, and BamHI, destroyed the ability of EBV DNA to induce early antigen. However, only SalI seemed to leave intact the full spectrum of antigen expression by the HR-1 and FF41 strains of EBV DNA. By means of transfection with recombinant DNA plasmids containing different EBV (FF41) DNA fragments generated by EcoRI, we showed that the coding region for early antigen was at least partially contained on the 17.2-megadalton EcoRI B fragment. (Author abstract) (22 Refs)

24. THE SHOPE PAPILLOMA-CARCINOMA COMPLEX OF RABBITS: A MODEL SYSTEM OF NEOPLASTIC PROGRESSION AND SPONTANEOUS REGRESSION.

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Adv Cancer Res; 35:81-110 1981

Shope papilloma virus (SPV), an indigenous disease in rabbits of the high plains of the United States that is transmissible to other rabbits, is discussed as a model of neoplastic progression and spontaneous regression. Topics include historical origins of the model, the interaction of SPV and host cells (natural occurrence and transmission, virion and genome structure, factors influencing SPV replication, the rabbit kidney vacuolating virus, host cell specificity, cell and tissue culture studies, papilloma arginase), neoplastic progression (malignant transformation, factors influencing neoplastic progression, the role of SPV genome in carcinomatous transformation), and spontaneous regression (growth, genetic and immunological factors in spontaneous regression). Theoretical and practical aspects of the Shope papilloma-carcinoma complex as a model system are advanced; the relevance to human neoplasia is considered. (123 Refs)

25. EXPRESSION OF HERPES SIMPLEX VIRUS COMMON SURFACE ANTIGENS AND MALIGNANCY BY CLONAL CELLS OF A HERPES SIMPLEX VIRUS TYPE 2-TRANSFORMED LINE.

Kimura S, Okazaki K, Tanaka S, Yoshida N
Dept. Microbiology, Kochi Medical Sch., Oko-cho, Nankoku-shi, Kochi-ken 781-51, Japan
Gan; 72(6):834-841 1981

Herpes simplex virus (HSV) type common surface antigens (CSA) were examined by indirect immunofluorescence with rabbit antiserum to HSV Type 1 in a clonal hamster cell line 155-4 transformed by HSV Type 2. The tumor formation was examined in hamsters transplanted with various transformed and tumor cells. The examination of subclones derived from 155-4 cell line gave the following results. (1) Thirty subclones were isolated and classified into three phenotypes as to CSA expression: (i) in CSA-positive type (20% of the clones isolated), the number of CSA-positive cells increased soon (5 hr) after seeding at 37 C; (ii) in CSA-inducible type (33% of the clones), the number of CSA-positive cells increased after treatment with actinomycin D (ACT-D, 2 ug/ml), but not without ACT-D; (iii) in CSA-negative type (47% of the clones), the number of CSA-positive cells did not increase after seeding or after ACT-D treatment. (2) In tumor cell lines derived from the parent line and from three representative clones, CSA expressions were similar to that by CSA-negative type. (3) Transformed cells expressing CSA after seeding or after ACT-D treatment formed tumors and metastases less efficiently than cells expressing little CSA in transplanted hamsters. (4) Tumor cell lines formed tumors and metastases more efficiently than transformed cells expressing little CSA in transplanted hamsters. (Author abstract) (17 Refs)

VIRUS REPLICATION AND EXPRESSION

26. SPECIFIC IN VITRO INITIATION OF TRANSCRIPTION ON THE ADENOVIRUS TYPE 2 EARLY AND LATE EII TRANSCRIPTION UNITS.

Mathis DJ, Elkaim R, Kedinger C, Sassone-Corsi P, Chambon P
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Proc Natl Acad Sci USA; 78(12):7383-7387 1981

Three transcription units are present in the adenovirus Type 2 region EII. Transcription units EIIaE and EIIaL encode the mRNA for the 72,000-dalton DNA binding protein, early and late in the lytic cycle, respectively, and transcription unit EIIb encodes the mRNA for the protein that binds to the 5' termini of adenovirus DNA. By using a cell-free transcription system in the presence of purified RNA polymerase B (or II), we have obtained specific initiation of transcription from both the EIIaE p romoter, which does not contain a T-A-T-A box, and the EIIaL promoter, which does. In addition, we have identi-

fied a new EII T-A-T-A box promoter that is located close to the early non-T-A-T-A box promoter and is used both in vivo and in vitro. (Author abstract) (28 Refs)

27. ASSEMBLY OF ADENOVIRUS PENTON BASE AND FIBER.

Boudin ML, Boulanger P
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Virology; 116(2):589-604 1982

Human adenovirus Type 2 (H2) fiber isolated from the cellular pool of H2 wild-type (WT) soluble components was found to be capable of assembly in vitro with penton base obtained from the fiber-defective temperature-sensitive (ts) mutant H2 ts-125. This assembly occurred over a relatively broad range of pHs (5.5-9.0) and of ionic strengths (0.05-1.0). The extent of penton formation was estimated by two-dimensional immunoelectrophoresis. The legitimacy of the assembly in vitro was demonstrated by morphological, biochemical, and biological evidence. The assembly of the penton base with the fiber appeared to be a reversible reaction, with a dissociation constant KD = 2x10(-7) M, in terms of fiber molarity. This value implied a high affinity of the penton base for the fiber. Comparative digestions by carboxypeptidase of uncombined fiber and of penton base-combined fiber suggested that the recognition site for assembly involved the last 20 amino acids of the C-terminal sequence of the fiber polypeptide chain. The same analysis performed on [14C]glucosamine-labeled fiber and penton seemed to indicate that the carbohydrate units carried by the fiber were positioned too far from the C-end to be directly involved in the recognition and assembly process of the penton base with the fiber. Chimeric penton produced in vitro by incubating fiber and penton base from different adenovirus serotypes, as well as in vivo by interserotypic ts+ recombinants suggested a group specificity of the recognition site on the fiber and a likely highly conserved C-terminal amino acid sequence. Assembly of penton base and fiber in vitro resulted in a significant change in the antigenicity of the penton base-combined fiber, which was thought to reflect some three-dimensional remolding. (Author abstract) (25 Refs)

28. TRANSFORMATION-DEFECTIVE MUTANT OF ADENOVIRUS TYPE 5 CONTAINING A SINGLE ALTERED E1A MRNA SPECIES.

Carlock LR, Jones NC
Dept. Biological Sciences, Purdue Univ., West Lafayette, IN, 47907
J Virol; 40(3):657-664 1981

A mutant of adenovirus type 5 containing an octanucleotide insert in region E1a of the viral genome was constructed. The insert was present in only one (13s) of the three overlapping mRNA's synthesized from this region. The insert was within the sequences removed by RNA splicing during the production of the other two mRNA's. The insertion resulted in a shift in the translational reading frame of the 13s mRNA and the probable premature termination of translation. The mutant was defective for viral DNA replication in HeLa cells and the transformation of rat embryo and baby rat kidney cells, indicating that a product encoded by the 13s mRNA is required for these two processes. Other early regions of the genome were expressed in HeLa cells infected by this mutant although in some cases the expression was decreased as compared with wild-type-infected cells. (Author abstract) (27 Refs)

29. DNA SEQUENCES AFFECTING SPECIFIC INITIATION OF TRANSCRIPTION IN VITRO FROM THE EIII PROMOTER OF ADENOVIRUS 2.

Lee DC, Roeder RG, Wold WS
Dept. Pharmacology SJ-30, Univ. Washington, Seattle, WA, 98195
Proc Natl Acad Sci USA; 79(1):41-45 1982

We have identified those sequences affecting the level of specific initiation of transcription in vitro from the EIII promoter of adenovirus 2. Mutants containing deletions in and around the initiation sites were constructed in cloned viral DNA fragments and assayed for their ability to initiate transcription in vitro. Three classes of mutants were studied with deletions in the following regions:-38 to-268,-21 to-71 (which includes the T-A-T-A-A box), and-29 through the cap sites (+1 and +3). Deletions that remove some or all of the area from-28 to several nucleotides downstream from the cap sites essentially abolished specific transcription. Small deletions in the region-30 to-41 reduced transcription to approx 60% of wild type; larger deletions in the region-35 to-268 reduced transcription to 30-40% of wild type. Deletions beginning from approximately +10 to +25 and extending further downstream reduced transcription to 20-40% of wild type, whereas a deletion beginning at +31 had little or no effect. Our results suggest that the region including the T-A-T-A-A box and extending to the area immediately beyond the cap sites is essential for specific transcription in vitro from the EIII promoter. However, sequences upstream from the T-A-T-A-A box and those downstream from the cap sites appear to significantly modulate the levels of transcription. (Author abstract) (26 Refs)

30. IDENTIFICATION OF ADENOVIRUS 2 EARLY REGION 4 POLYPEPTIDES BY IN VITRO TRANSLATION AND TRYPTIC PEPTIC MAP ANALYSIS.

Matsuo T, Hashimoto S, Wold WS, Symington J, Rankin A, Green M
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J Virol; 41(1):334-339 1982

The mRNA species encoded by early region 4 (E4) [map position (mp) 91.5 to 99.3] of adenovirus 2 were isolated from the polysomes of infected KB cells and were purified by hybridization to the cloned HindIII-F fragment (mp 89.5 to 97.3) or to EcoRI-C fragment (mp 89.7 to 100). The mRNA's w ere translated in vitro using [35S]methionine as a labeled precursor in rabbit reticulocyte lysates treated with micrococcal nuclease as well as in wheat germ lysates. Five major (35,000-mol-wt [35K], 23K, 22K, 21K, 18K) polypeptides were observed when the reticulocyte lysate was used. The 23K, 22K, 21K, and 18K polypeptides were also observed with the wheat germ lysate, as well as a very prominent 11K polypeptide; the 35K polypeptide was not observed. Assignment of these polypeptides to E4 was further established by hybrid arrested translation. Two-dimensional gel electrophoresis of a wheat germ translate resolved five polypeptides ranging from 18K to 23K, the major 11K polypeptide, and polypeptides of 10K and 9K. The in vitro 23K to 18K and 11K polypeptides migrated to approx the same positions on two-dimensional gels as did seven 26K to 21K polypeptides and an 11K polypeptide synthesized in vivo. Two-dimensional tryptic peptide maps demonstrated that the 35K, 23K, 22K, 21K, and 18K polypeptides are related. The peptide map of 11K is different from those of the above polypeptides, although 11K may share one tryptic methionine polypeptide with them. These results indicate that E4 encodes a major 11K polypeptide, as well as major 35K, 23K, 22K, 21K, and 18K polypeptides. (Author abstract) (23 Refs)

31. INITIATION OF ADENOVIRUS DNA REPLICATION: DETECTION OF COVALENT COMPLEXES BETWEEN NUCLEOTIDE AND THE 80-KILODALTON TERMINAL PROTEIN.

Challberg MD, Ostrove JM, Kelly TJ
Dept. Molecular Biology and Genetics, Johns Hopkins Univ. Sch. Medicine, Baltimore, MD, 21205
J Virol; 41(1):265-270 1982

We have previously shown that the 5'-terminal deoxycytidine residue of each nascent adenovirus 5 DNA strand synthesized in vitro is covalently linked to the 80-kilodalton (kd) terminal protein precursor via a phosphodiester bond to a serine residue in the protein. When extracts prepared from adenovirus 5-infected cells are incubated with [alpha-32P]dCTP as the only added deoxynucleoside triphosphate, complexes consisting of nucleotide covalently linked to the 80-kd protein can be detected. The nucleotide moieties present in such complexes include d(pC) and d(pCpA), the 5'-terminal nucleotide and dinucleotide of adenovirus 5 DNA, respectively, as well as some longer oligonucleotides. The formation of these complexes requires the presence of adenovirus DNA containing the attached 55-kd terminal protein and ATP. Extracts from H5ts125-infected cells which are defective in DNA replication catalyze complex formation to the same extent as adenovirus-coded 72-kd DNA-binding protein is apparently not required. Most, if not all, of the 80-kd protein-nucleotide complexes that are formed are noncovalently bound to the input viral DNA. These observations are consistent with the protein-priming model for the initiation of adenovirus DNA replication. (Author abstract) (25 Refs)

32. SECONDARY STRUCTURE MODEL FOR THE COMPLETE SIMIAN VIRUS 40 LATE PRECURSOR MRNA.

Nussinov R, Tinoco I, Jacobson AB
Dept. Chemistry, Univ. California at Berkeley, Berkeleyook, CA, 94720
Nucleic Acids Res; 10(1):351-363 1982

Structures for all sequences containing less than 1,790 nucleotides in the 2,600 nucleotide late region of the SV40 virus have been computed and saved on magnetic tape. Previously the longest sequence whose secondary structure was calculated in a single computer run contained 950 nucleotides. In the past, analysis of long molecules required numerous repeated, partially overlapping computations on much shorter segments. The structure obtained for the late half of the SV40 is Y-shaped with two unequal arms. It has 52 short hairpins. Two long range interactions between nucleotides near 650 and 1,350 and between 1,450 and 2,450 appear to play an important role. The first is within the 16S intron; the second is in the 3' exon. The 5' and 3' ends of the molecule are close to each other and are found in the major elongated stem in the vicinity of the fork. (Author abstract) (30 Refs)

33. DNA AND HISTONE SYNTHESIS IN BUTYRATE-INHIBITED BSC-1 CELLS INFECTED WITH SV40.

Daniell E, Burg JL, Fedor MJ
Dept. Molecular Biology, Univ. California, Berkeley, CA, 94720
Virology; 116(1):196-206 1982

The effect of butyrate on DNA synthesis, histone synthesis, and virus production in SV40-infected monkey cells has been studied. When cells in which DNA and histone synthesis have been blocked by treatment with butyrate are infected with SV40, DNA synthesis is induced to a level at least equal to that in untreated infected cells. Both viral and cellular DNA sequences are replicated. Viral DNA synthesized is predominantly SV40 Form I. The amount of viral DNA which accumulates is reduced by 10-fold in the presence of butyrate, and the yield of infectious virus is lowered 100-fold. Histone synthesis in these cells is stimulated concomitantly with DNA synthesis. The hyperacetylation of histones characteristic of butyrate-treated cells is unchanged by SV40 infection; the newly synthesized histones are hyperacetylated. Thus the mechanism by which butyrate blocks DNA and histone synthesis is not directly related to the hyperacetylation of histones. (Author abstract) (30 Refs)

34. MAPPING THE IN VIVO ARRANGEMENT OF NUCLEOSOMES ON SIMIAN VIRUS 40 CHROMATIN BY THE PHOTOADDITION OF RADIOACTIVE HYDROXYMETHYLTRIMETHYLPSORALEN.

Robinson GW, Hallick LM
Dept. Biochemistry, Oregon Health Sciences Univ., Portland, OR, 97201
J Virol; 41(1):78-87 1982

Intracellular simian virus 40 (SV40) chromatin was photoreacted with a 3H-labeled psoralen derivative, hydroxymethyltrimethylpsoralen (HMT), at 48 hr postinfection. Psoralen compounds have been shown to readily penetrate intact cells and, in the presence of long-wavelength UV light, form covalent adducts to DNA, preferentially at regions unprotected by nucleosomes. The average distribution pattern of [3H]HMT on the SV40 genome was determined by specific activity measurements of the DNA fragments generated by HindIII plus HpaII or by AtuI restriction enzyme digestion. At levels of 1 to 10 [3H]HMT photoadducts per SV40 molecule, the radiolabel was found to be distributed nonrandomly. Comparison of the labeling pattern in vivo with that of purified SV40 DNA labeled in vitro revealed one major difference. A region of approx 400 base pairs, located between 0.65 and 0.73 on the physical map, was preferentially labeled under in vivo conditions. This finding strongly suggests that the highly accessible region near the origin of replication, previously observed on isolated SV40 'minichromosomes,' exists on SV40 chromatin in vivo during a lytic infection. (Author abstract) (66 Refs)

35. PREREPLICATIVE EVENTS INVOLVING SIMIAN VIRUS 40 DNA IN PERMISSIVE CELLS.

Rinaldy A, Feunteun J, Rosenberg BH
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J Virol; 41(1):237-243 1982

Simian virus 40 DNA molecules were found to be unable to replicate for 9 hr after infection, even in cells that were already replicating the DNA of preinfecting simian virus 40; after 9 hr, the ability of the DNA to replicate began to rise sharply. The kinetics of activation indicated that each DNA molecule undergoes a series of slow consecutive reactions, not involving T-antigen, before it can replicate. These pre-replicative molecular transformations probably involve configurational changes; their nature and their relation to the initiation of viral DNA synthesis is discussed. Observation of the replicative behavior of one viral DNA in the presence of another was made possible by the use of two different mutants with distinguishable DNAs: a viable deletion mutant containing DNA insensitive to TaqI restriction enzyme was used to provide viral functions required for replication, and a tsA mutant with TaqI-sensitive DNA was introduced at various times as a probe to

determine the ability of the DNA to replicate under different conditions. (Author abstract) (20 Refs)

36. REGULATION OF SV40 GENE EXPRESSION.

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Adv Cancer Res; 35:111-149 1981

The microinjection approach to the study of simian virus 40 (SV40) and polyoma virus (PV) gene expression is discussed. The technique permits placing of biological material from virtually any source directly into living, single-tissue culture cells using glass micropipettes. Cell type dependence of SV40 and PV gene expression is presented in relation to permissive, nonpermissive, semi-permissive, and virus-resistant cells. Functions of SV40 tumor antigens are considered, including induction of cellular and viral DNA systems, regulation of late and early SV40 gene expression, stimulation of rRNA synthesis, helper function for adenovirus and U antigenicity, and reduction of the actin cable structure. Experiments with microinjection of SV40 DNA fragments to induce cellular transformation and applications of microinjection are reviewed. (78 Refs)

37. PATHWAY OF ASSEMBLY OF HERPESVIRUS CAPSIDS: AN ANALYSIS USING DNA+ TEMPERATURE-SENSITIVE MUTANTS OF PSEUDORABIES VIRUS.

Ladin BF, Ihara S, Hampl H, Ben-Porat T
(c/o Ben-Porat) Dept. Microbiology, Vanderbilt Univ. Sch. Medicine, Nashville, TN, 37232
Virology; 116(2):544-561 1982

It was reported previously that nine different DNA+ ts mutants of Pr virus were all defective in the processes leading to the cleavage of concatemeric viral DNA and to the formation of nucleocapsids. Six of these mutants were found to assemble capsids at the nonpermissive temperature; three were capsid-. In the present communication we have characterized these mutants further. Each of the mutants was rescued by one specific restriction fragment of wild type virus DNA indicating that none of these mutants is altered in more than one locus. Many of the mutants complemented one another well at a frequency indicating that they are probably mutated in different genes. Cells infected at the nonpermissive temperature (41 C) with the DNA+ mutants synthesized all the virus proteins synthesized by wild type-infected cells (as detected by PAGE) with two notable exceptions: (1) ts1-infected cells synthesized a thermolable 142K capsid protein. (2) In ts1, ts109, and tsJ (all capsid-mutants)-infected cells, detectable amounts of 35K capsid protein did not accumulate. The 35 K capsid protein is normally processed from a precursor protein, the processed protein being detectable in the nucleus of the infected cells only. In capsid- infected cells processing of the 35K protein precursor did not occur. Furthermore, accumulation in the nucleus of all capsid proteins was impaired in all the capsid-infected cells. Because at least two of the three capsid- mutants are mutated in different genes, we conclude that the processing of the 35K protein, capsid assembly, and accumulation of capsid proteins in the nuclei are interdependent events which occur only if all the proteins necessary for capsid assembly are functional. On the basis of these results, we propose that the 35K protein is processed during capsid assembly and the assembly of the proteins into capsids is required for the continued movement of capsid proteins to, and their accumulation in, the nuclei of infected cells. (Author abstract) (38 Refs)

38. ACTIVATION OF HERPES SIMPLEX VIRUS (HSV) TYPE 1 GENOME BY TEMPERATURE-SENSITIVE MUTANTS OF HSV TYPE 2.

Wigdahl BL, Isom HC, De Clercq E, Rapp F
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Virology; 116(2):468-479 1982

Previous studies have demonstrated that herpes simplex viruses (HSV) Type 1 (HSV-1) and Type 2 (HSV-2) can be maintained in a repressed form in human embryo lung cells. Reducing the incubation temperature or superinfecting with a heterologous herpesvirus, human cytomegalovirus (HCMV), results in activation of virus replication. We now report that superinfection with a partially homologous herpesvirus, HSV-2, also resulted in activation of HSV-1. To minimize excessive synthesis of infectious HSV-2 while allowing virus gene expression, repressed HSV-1-infected cultures were superinfected with HSV-2 temperature-sensitive (ts) mutants (tsF3, tsB5, or tsH9). The predominant virus replicated after HSV-2 ts mutant superinfection at a nonpermissive temperature was identified as activated parental-like HSV-1 by (i) plaquing efficiency at permissive (34 C) and nonpermissive (40.5 C) temperatures, (ii) sensitivity to inhibition by the HSV-1-specific antiviral agent (E)-5-(2-bromovinyl)-2'-deoxyuridine, and (iii) restriction endonuclease cleavage analysis. In addition, the fact that superinfection with HSV-2 tsB5 or tsH9, which are unable to synthesize virus DNA and express only early virus genes at nonpermissive temperature, resulted in synthesis of virus demonstrated that HSV-2 DNA synthesis is not required for activation. This system has provided the basis for further studies concerning the regulation of HSV gene expression in human cells. (Author abstract) (31 Refs)

39. THE EFFECT OF A TEMPERATURE-SENSITIVE LESION IN THE ALKALINE DNASE OF HERPES SIMPLEX VIRUS TYPE 2 ON THE SYNTHESIS OF VIRAL DNA.

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Dept. Human Genetics, Yale Univ. Medical Sch., 333 Cedar St., New Haven, CT, 06510
Virology; 116(1):116-127 1982

The alkaline DNase of herpes simplex virus Type 2 appears to be a nonessential function, since revertants of the original ts13 mutant selected for growth at nonpermissive temperature, have reverted only in the thermostability of the virus particle while still inducing a temperature-sensitive DNase. The properties of one revertant, 4-8, are investigated in this report. During infection at 38.5 C production of infectious viral is reduced ca 10-fold and 4-8 forms tiny plaques on monolayers when compared to 31.5 C or to wild type virus at either temperature, where normal plaque sizes are observed. 4-8 is DNA+/- at 38.5 C, producing 5 to 10% of the viral DNA made at 31.5 C or by wild type virus at either temperature, similar to the DNA phenotype of the original ts13. DNase activity is absent from 4-8 infected cells at 38.5 C and DNA polymerase levels are reduced four- to five-fold. Although the viral DNA polymerase, when assayed in extracts from cells infected at 31.5 C is more heat stable in 4-8 than in wild type, the DNA polymerase induced by 4-8 at 31.5 C is temperature sensitive in vivo, when infected cells are shifted from 31.5 C to 38.5 C. The drop in DNA polymerase activity under these conditions is paralleled by a decrease in the synthesis of viral DNA. But in contrast to the DNA- mutant ts6, where DNA synthesis turns off completely in response to a temperature shift-up, 4-8 continues to synthesize viral DNA for several

hours after shift-up at a 10-to 20-fold reduced rate. The results demonstrate that viral DNA is synthesized in the absence of active DNase, yet less efficiently so than in its presence. The possibility that the DNase is responsible for the generation of rapidly sedimenting, concatemeric viral DNA can be excluded since the same proportion of pulse-labeled DNA appears in this fraction for 4-8 as for wild type independent of the temperature. (Author abstract) (19 Refs)

40. SHOPE FIBROMA VIRUS II. ROLE OF THE VIRION-ASSOCIATED NUCLEASES.

Pogo BG, Stein A, Freimuth P
Center for Experimental Cell Biology, Mount Sinai Sch. Medicine, New York, NY, 10029
J Virol; 41(1):104-109 1982

The effect of Shope fibroma virus (SFV) infection on host DNA synthesis was investigated. The cytocidal strain, SFV-I, inhibited the incorporation of [3H]thymidine into nuclear DNA very shortly (2 hr) after infection, whereas the noncytocidal strain, SFV-W, did so later (10 hr postinfection) and to a lesser extent. Furthermore, a two- to threefold stimulation of host DNA synthesis was recorded in SFV-W-infected cells 3 to 4 hr after infection. Since virion-associated nucleases have been implicated in the shutoff of host synthesis, these and other enzymatic activities were measured in purified virion preparations. The SFV strains and vaccinia virus contained equivalent amounts of DNA-dependent RNA polymerase, ATPase, and protein kinase activities. However, in SFV-W the pH 4.5 exonuclease activity was lower than in SFV-I and vaccinia virus, and the level of pH 7.8 endonuclease was almost undetectable. To test whether the lack of endonucleolytic activity had some effect on the removal of the cross-links in the parental DNA that occurs after viral penetration, the fate of the virion SFV DNA was followed. The majority (80%) of the SFV-I and SFV-W DNA molecules extracted after viral adsorption sedimented in alkaline sucrose gradients as cross-linked. After 3 hr of infection, 75% of the SFV-I DNA molecules lacked cross-links, whereas 78% of the SFV-W DNA still remained cross-linked. The same results were obtained when the presence of cross-links was tested in restriction fragments. Taken together, these results indicate that virion-associated nucleases are involved in the early shutoff of host DNA synthesis and in the elimination of cross-links from the parental viral DNA. (Author abstract) (29 Refs)

41. EPSTEIN-BARR VIRUS-RELATED DNA-BINDING PROTEINS INDUCED BY N-BUTYRATE IN P3HR-1 CELLS.

Sugawara K, Kawanishi M, Ito Y
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Virology; 116(1):354-358 1982

Epstein-Barr virus (EBV)-related early and late proteins induced in P3HR-1 cells by n-butyrate treatment were examined for DNA-binding ability using affinity chromatography on native and denatured DNA-cellulose. At least 5 (140K, 120K, 50K, 40K and 35K) of 16 early polypeptides and 3 (145K, 46K and 18K) of 6 late polypeptides were identified as DNA-binding proteins, and most of them bound to both native and denatured DNA. The results indicate that EBV-induced products contain proteins involved in viral DNA replication and in encapsidation. (Author abstract) (28 Refs)

VIRAL GENETICS

42. MOLECULAR GENETICS OF HERPES SIMPLEX VIRUS. V. CHARACTERIZATION OF A MUTANT DEFECTIVE IN ABILITY TO FORM PLAQUES AT LOW TEMPERATURES AND IN A VIRAL FUNCTION WHICH PREVENTS ACCUMULATION OF CORELESS CAPSIDS AT NUCLEAR PORES LATE IN INFECTION.

Tognon M, Furlong D, Conley AJ, Roizman B
Inst. Microbiology, Univ. Ferrara, Ferrara, Italy
J Virol; 40(3):870-880 1981

In herpes simplex virus-infected cells, coreless capsids accumulate at the nuclear pores soon after infection, but subsequently disappear, suggesting that, as in adenovirus-infected cells, the release of viral DNA from nucleocapsids takes place at the nuclear pores. A nonlethal mutant, HSV-1(50B), produced by mutagenesis of HSV DNA fragments and selected for delayed production of plaques at 31 C, accumulated coreless capsids at the nuclear pores late in infection in contrast to wild-type viruses. Recombinants selected for ability to produce plaques at 31 C by marker rescue with digests of herpes simplex virus 2 DNA and selected cloned fragments of HSV-1 DNA no longer accumulated empty capsids at nuclear pores late in infection. These results suggest that herpes simplex viruses encode a function which prevents accumulation of coreless capsids at nuclear pores, presumably by preventing uptake, unenvelopment, and DNA release from progeny virus, and indicate that the cold sensitivity of plaque formation and accumulation of coreless capsids might be related or comap in the S component of the genome. (Author abstract) (28 Refs)

43. COMPLEMENTATION BETWEEN PHOSPHONOACETIC ACID-RESISTANT AND -SENSITIVE VARIANTS OF HERPES SIMPLEX VIRUSES: EVIDENCE FOR AN OLIGOMERIC PROTEIN WITH RESTRICTED INTRACELLULAR DIFFUSION AS THE DETERMINANT OF RESISTANCE AND SENSITIVITY.

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Div. Virology, Natl. Inst. Medical Res., Mill Hill, London NW7 1AA, England
J Gen Virol; 57(part2):297-306 1981

Complementation between phosphonoacetic acid (PAA)-resistant (Pr) and -sensitive (Ps) variants of herpes simplex virus Type 1 (HSV-1) and Type 2 (HSV-2) was studied to provide information on the function of the virus-coded DNA polymerase. Complementation within and between serotypes was demonstrated, with the growth of the Ps partner in mixed infections becoming relatively more resistant and the Pr partner relatively more sensitive to PAA than in the corresponding single infections. However, the relative contribution of the Ps partner to the mixed infection had a disproportionately large effect on the resultant sensitivity of the mixed infection which was incompatible with non-interactive (eg monomeric) polymerase molecules as determinants of PAA sensitivity and resistance. Although a number of solutions gave equally good fits to the available data, the simplest was obtained by assuming that the functional DNA polymerase was a trimer and that only the (Pr)(3) homotrimer was active in the presence of the drug. In addition, yields from mixed infections in the presence of PAA were enriched for the resistant partner relative to yields in the absence of the drug. These latter results suggested that the intracellular distribution of resistant DNA polymerase oligomers was non-random with respect to resistant and sensitive tem-

plate genomes and these resistant polymerase molecules were more likely to encounter and replicate resistant than sensitive genomes. Such an explantation seems to require vectorial nuclear-cytoplasm-nucleus translocation and restricted diffusion of transcript and gene products determining resistance. (Author abstract) (19 Refs)

IMMUNOLOGICAL STUDIES

44. NATURAL CYTOTOXICITY TO MURINE CYTOMEGALOVIRUS-INFECTED CELLS MEDIATED BY MOUSE LYMPHOID CELLS: ROLE OF INTERFERON IN THE ENDOGENOUS NATURAL CYTOTOXICITY REACTION.

Lee GD, Keller R
Dept. Microbiology, Michael Reese Hosp. and Medical Center, Chicago, IL, 60616
Infect Immun; 35(1):5-12 1982

Lymphoid cells from unstimulated normal C57BL/6J mice were shown to lyse murine cytomegalovirus (MCMV)-infected syngeneic mouse embryo fibroblasts but not uninfected mouse embryo fibroblasts. This cytotoxicity by mouse effector cells was not restricted to MCMV-infected syngeneic cells. The effector cells mediating this cytotoxicity against MCMV-infected cells are similar to described natural killer (NK) cells mediating lysis of tumor cells and virus-infected cells. Because of the described augmentation of NK activity by interferon, we examined the role of interferon in the NK reaction. Although low levels of virus-induced interferon were detectable in supernatants of MCMV-infected mouse embryo fibrobl asts, no interferon was detectable in supernatants of MCMV-infected rat heart fibroblasts, a target significantly more sensitive to NK cytolysis than infected mouse embryo fibroblasts. We were able to augment the NK reaction against MCMV-infected cells by in vitro treatments with interferon. The amounts of interferon required for augmentation were significantly greater than the amounts generated by infected target cells. In vitro interferon-stimulated NK cells retained selective cytotoxic activity since they continued to remain incapable of lysing uninfected target cells. MCMV-infected rat heart fibroblasts induced more interferon and were also more susceptible to NK activity than MCMV-infected mouse embryo fibroblasts. In spite of this difference in interferon-inducing capacity, there was no augmentation of cytotoxicity of MCMV-infected mouse embryo fibroblasts when mouse splenocytes were cocultivated with both target cells. Finally, when production of interferon in the NK reaction was inhibited by the addition of actinomycin D, no reduction of NK activity was seen. Our findings suggest that native mouse NK cells can discriminate between MCMV-infected cells and uninfected cells, this ability leading to the selective lysis of the virus-infected cells. Furthermore, although we could demonstrate augmentation of NK activity by interferon, interferon activation of NK cells may not be a necessary precondition for the development of endogenous NK activity. (35 Refs)

45. FUNCTIONAL CHANGES OF MACROPHAGES FROM POLYOMA TUMOR-BEARING MICE.

Pituch-Noworolska A, Pajdak E
Div. Clinical Immunology and Microbiology, Inst. Pediatrics, Wielicka 265, 30-663 Krakow, Poland
Arch Immunol Ther Exp (Warsz); 29(4):423-429 1981

CBA mice were inoculated with different doses of polyoma tumor cells. The PHA response of spleen cells from tumor-bearers was decreased but the spontaneous incorporation of 3H-leucine was increased. PHA-induced cytotoxicity of spleen cells was increased only in tumor-bearers. The expression of Fc receptors showed a slight increase on adherent cells from spleen and peripheral blood mononuclear cells under suboptimal conditions for EA rosette formation (small amount of antibody for coating the erythrocytes). The non-specific esterase activity was assayed in an extract of peritoneal macrophages and the elevated activity with isoenzymes was seen in a group of tumor-bearers. The results suggest a functional alteration of macrophages in polyoma tumor-bearing mice. (Author abstract) (24 Refs)

46. INTERACTION OF HERPES SIMPLEX VIRUS WITH HUMAN CELL LINES AT VARIOUS STAGES OF LYMPHOID DIFFERENTIATION.

Katz E, Mitrani-Rosenbaum S, Margalith E, Ben-Bassat H
(c/o Ben-Bassat) Dept. Hematology, Hebrew Univ.-Hadassah Univ. Hosp. and Medical Sch., Jerusalem, Israel
Intervirology; 16(1):33-42 1981

The growth of herpes simplex virus (HSV) Type 1 in human lymphoid cell lines, arrested at various stages of differentiation, was studied. The synthesis of viral DNA and antigens and the production of infectious virus were followed in null, B and T leukemia-lymphoma cell lines. It was found that while 'nondifferentiated' null cells and cells differentiating along the B pathway, even at very early stages of differentiation, supported HSV growth, cell lines of T origin at all stages of differentiation were generally nonpermissive. The failure of T cells to support the growth of HSV did not result from inefficient adsorption to the host cell. However, viral DNA synthesis was not detected, and a significant reduction in the ability to synthesize HSV antigens was observed. It is suggested that the block in the growth cycle of the virus occurs at a stage after adsorption of the virion to the cells, but prior to synthesis of its DNA and proteins. (Author abstract) (22 Refs)

47. EFFECT OF THYMECTOMY ON DEVELOPMENT OF LUCKE RENAL ADENOCARCINOMAS IN VIRUS-INFECTED LEOPARD FROG TADPOLES.

Rollins-Smith LA, Cohen N
Dept. Microbiology, Div. Immunology, Univ. Rochester Medical Center, Sch. of Medicine and Dentistry, 601 Elmwood Ave., Rochester, NY, 14642
JNCI; 68(1):133-138 1982

Thymectomy of very young leopard frog (Rana pipiens) larvae dramatically reduced in vitro lymphocyte responses to the mitogen phytohemagglutinin-P and prolonged survival of Lucke tumor alloimplants. However, tumor incidence in premetamorphic or metamorphosing frogs that had received injections as embryos of Lucke tumor herpesvirus (LTHV) was the same in thymectomized and normal immunocompetent animals. This result suggests that T-cell-mediated cytotoxicity does not prevent development of Lucke tumors in immunocompetent larvae and that if tumor-specific transplantation antigens appear, they may induce tolerance rather than destructive im-

munity in normal LTHV-infected larvae. (Author abstract) (37 Refs)

48. IN-VIVO MODULATION OF MACROPHAGE FUNCTIONS BY HERPES SIMPLEX VIRUS TYPE 2 IN RESISTANT AND SENSITIVE INBRED MOUSE STRAINS.

Armerding D, Mayer P, Scriba M, Hren A, Rossiter H
Sandoz Forschungsinstitut G.m.b.H., Brunner Strasse 59, A-1235 Vienna, Austria
Immunobiology; 160(2):217-227 1981

Intra-peritoneal (ip) infection of mice with herpes simplex virus Type 2 (HSV 2) attracted macrophages into the peritoneum. Macrophages from moderately and highly HSV 2 resistant mouse strains expressed elevated phagocytosis activity 24 hours after injection. Stimulation of phagocytosis in low resistant strains was generally less effective or absent. This was, in some experiments, due to the fact that macrophages were already highly activated before the experimental infection. Ip infection also caused HSV replication in the adherent peritoneal exudate cell (PEC) population. The capacity of macrophages supporting HSV 2 replication was low in three of four resistant mouse strains and high in all moderately and highly susceptible and in one of the resistant (SJL) strains when determined 24 hours after infection. Four different F1 hybrids between resistant and susceptible strains exhibited significantly lower yields of virus-producing macrophages than the HSV-sensitive parent. One hybrid between two HSV-susceptible lines restricted virus replication in the PEC population better than both parental strains. (Author abstract) (25 Refs)

OTHER RELATED STUDIES

49. ESTABLISHMENT OF MACROPHAGE-LIKE CELL LINES OF THE GUINEA PIG.

Kato K, Katsuka T, Ohnuki T, Tokunaga T
Dept. Enteroviruses, Natl. Inst. Health, Kamiosaki, Shinagawa-ku, Tokyo, Japan
Microbiol Immunol; 25(11):1209-1214 1981

The establishment of three macrophage-like cell lines from adherent cells of peritoneum and bone marrow of strain 13 guinea pigs infected with SV40 and BK viruses is reported. These lines are adherent, phagocytic and secrete lysozyme. Two express guinea pig leukocyte antigens while one does not. (17 Refs)

50. CELLULAR MOLONEY MURINE SARCOMA (C-MOS) SEQUENCES ARE HYPERMETHYLATED AND TRANSCRIPTIONALLY SILENT IN NORMAL AND TRANSFORMED RODENT CELLS.

Gattoni S, Kirschmeier P, Weinstein IB, Escobedo J, Dina D
Div. Environmental Sciences, Inst. Cancer Res., Columbia Univ., New York, NY, 10032
Mol Cell Biol; 2(1):42-51 1982

The extent of methylation of the oncogenic v-mos sequence of Moloney murine sarcoma virus and its homologue present in normal cells of various vertebrate species (c-mos) was investigated. Results indicated that normal development and differentiation, prolonged serial passage, or transformation (either spontaneous or induced by radiation, chemical carcinogens, or polyoma virus) are not associated with gross rearrangements of the endogenous c-mos sequences in cellular DNA. The c-mos sequence does not contain any intervening sequences and is practically identical to the v-mos sequence. C-mos was not transcribed into RNA in any cells studied. These studies do not rule out the possibility that transient expression of c-mos sequences did occur during early transformation, or that other cellular oncogenic genes might play a role in cell transformation induced by chemical carcinogens or radiation. (43 Refs)

51. SIMIAN VIRUS 40 AS A PROBE FOR STUDYING INDUCIBLE REPAIR FUNCTIONS IN MAMMALIAN CELLS.

Mezzina M, Gentil A, Sarasin A
Institut de Recherches Scientifiques sur le Cancer, B.P. no 8, 94802 Villejuif Cedex, France
J Supramol Struct Cell Biochem; 17(2):121-131 1981

Simian virus 40 (SV40) was used as a biological probe to examine inducible DNA repair pathways in monkey kidney cells in order to study the effect of pyrimidine dimer lesions on SV40 DNA replication, the induction of an error-prone replication process in DNA-damaged monkey cells, and the presence of recombinogenic activity in carcinogen-treated cells. Characteristics of viral replicative intermediate molecules made shortly after UV irradiation were studied in irradiated SV40-infected African green monkey cells. The tight homology between the size of newly synthesized DNA made after UV irradiation (40%), the av length of the tail measured in the same samples by electron microscopy analysis (41%), and the av distance between two pyrimidine dimers measured with the T4 endonuclease V (37%) suggested that the viral replication fork is blocked by a pyrimidine dimer leaving a single-stranded region in the opposite strand where an endonucleolytic cleavage may occur to generate the tail. Evidence for the induction of error-prone DNA replication is presented for SV40 cells in which DNA synthesis has been inhibited. An experimental protocol is proposed for the study of inducible recombination through the use of SV40 genetic markers. (36 Refs)

52. INTERFERON: IMMUNOBIOLOGY AND CLINICAL SIGNIFICANCE.

Stiehm ER, Kronenberg LH, Rosenblatt HM, Bryson Y, Merigan TC
Dept. Pediatrics, Univ. California Los Angeles Sch. Medicine, Los Angeles, CA, 90024
Ann Intern Med; 96(1):80-93 1982

Interferons are proteins elaborated by infected cells that protect noninfected cells from viral infection. These proteins produce a temporary 'antiviral state' by altering nucleotide metabolism and cytoplasmic enzyme induction. Interferons appear early after viral infection locally and systematically to limit spread of viral infection; they also affect cell differentiation, growth, surface antigen expression, morphologic findings, and immunoregulation. Several human disorders have diminished interferon production. Newborns have normal interferon alpha but deficient interferon gamma production. Infants with congenital infections may also have defects in interferon production. Immunosuppressed patients receiving transplants (marrow, heart, or kidney) have diminished interferon production, particularly immediately after transplant. Deficiencies of interferon have also been noted in Down's syndrome, cellular immunodeficiencies, uremia, malnutrition, and hematopoietic malignancy. Leukocyte interferon has been of therapeutic value in herpes zoster infections, in patients with cancer, and in patients with hepatitis B infection. Interferon has not been proved to help children with congenital cytomegalovirus or

rubella. Interferon can shrink lymphoid tumors, particularly non-Hodgkin's lymphoma. (Author abstract) (160 Refs)

53. POLYAMINES IN MAMMALIAN TUMORS PART 1.

Scalabrino G, Ferioli ME
Inst. General Pathology, Univ. Milan, 20133 Milan, Italy
Adv Cancer Res; 35:151-268 1981

The connection between polyamines (PA) and cancer in mammals is reviewed, and evidence of a relationship between PA and neoplastic disease is evaluated. Biochemical and metabolic aspects discussed include properties of the PA biosynthetic enzymes S-adenosyl-L-methionine, 5'-methylthioadenosine, catabolism of major PA in mammals, conjugation and excretion products, and natural antibodies of PA. Levels of PA and biosynthetic enzymes in experimental tumors and modifications of in vivo and in vitro metabolism of PA by chemical carcinogens and tumor promoters (single and repeated promoter administration, multistage carcinogenesis, mutagenic and antimutagenic properties of PA) are reviewed. PA levels in urine, serum, and erythrocytes of rats during chemical carcinogenesis or when bearing several experimental tumors are considered. Effects of oncogenic RNA and DNA viruses in biosynthesis, levels of PA in cells during virus-induced transformation, and the role of physical carcinogens in changes in PA biosynthesis and content of target tissues are reviewed. (894 Refs)

54. CELLULAR MUTATIONS AND DRUG RESISTANCE PROBED BY HERPES SIMPLEX VIRUS.

Campisi J, Pardee AB
Sidney Farber Cancer Inst., Boston, MA, 02115
J Cell Physiol; 109(3):469-480 1981

Inhibition of herpes simplex virus (type-1) (HSV-1) plaque formation in African green monkey kidney (Vero) cell lines occurred in varying degrees when cultures were exposed to the following drugs: thioguanine (TG), cytosine arabinoside (AraC), methotrexate (MTX), 5-fluorodeoxyuridine (5-FUdR), 5-fluorouracil (5-FU), and bleomycin (BLM). Viral immunofluorescence was substantially reduced when HSV-1-infected human foreskin fibroblasts strain FS2 were exposed to the six drugs, except in the case of BLM, which was cytotoxic and had no antiviral effect. Fluorescence studies showed that TG markedly decreased viral growth in normal FS2 cells while such growth was unaffected in Lesch-Nyhan strain fibroblasts, a strain genetically resistant to killing by TG. While thymidine kinase-deficient HSV-1 (TK[-]HSV-1) did not grow in the normal mouse fibroblast line A31 in the presence of 5-FUdR, a TK-deficient derivative of A31 permitted such growth at low to moderate doses of 5-FUdR. HSV-1-infected xeroderma pigmentosum (group A) (XPA) fibroblasts and FS2 cells were UV-irradiated with 100 microwatts/square cm; immunofluorescence showed that viral activity declined much faster with increasing UV dose in XPA than in FS2 cells. In another experiment TG was effective in reducing HSV-1 fluorescence in one strain of human neck squamous carcinoma cells (SCC-15), but was relatively ineffective in another strain (SCC-25). (21 Refs)

55. RECEPTOR ACTIVITIES FOR LOW-DENSITY LIPOPROTEIN AND ACETYLATED LOW-DENSITY LIPOPROTEIN IN A MOUSE MACROPHAGE CELL LINE (IC21) AND IN HUMAN MONOCYTE-DERIVED MACROPHAGES.

Traber MG, Defendi V, Kayden HJ
Dept. Medicine, New York Univ. Medicine, New York, NY, 10016
J Exp Med; 154(6):1852-1867 1981

Lipoprotein and cholesterol metabolism in the mouse macrophage cell line IC21 (Simian virus 40-transformed peritoneal macrophages) was compared with that of human monocyte-derived (HMD) macrophages. IC21 macrophages exhibited detectable low-density lipoprotein (LDL) receptor activity; they degraded acetylated LDL (AcLDL) with high affinity. Saturation curves of 125I-AcLDL degradation by IC21 macrophages and mouse peritoneal macrophages were virtually superimposable. The 125I-AcLDL binding site on IC21 macrophages recognized dextran sulfate: the rates of 125I-AcLDL and 125I-LDL degradation were reduced 5-and 10-fold, respectively, in the presence of 5 ug/ml dextran sulfate. IC21 macrophages showed lower levels of high-affinity LDL degradation and greater capacity to degrade AcLDL than did HMD macrophages. When IC21 cells were incubated with lipoprotein-depleted serum (LPDS), cellular cholesterol content was decreased and the rate of sterol synthesis was markedly increased. Data suggested that IC21 macrophages were able to use AcLDL cholesterol in place of endogenously synthesized cholesterol; LDL cholesterol also entered the cells but at a slower rate and thus did not provide cholesterol in sufficient amounts to inhibit cholesterol synthesis completely. Further evidence that uptake of LDL via the LDL receptor pathway does not provide sufficient cholesterol to the IC21 macrophages was gained from comparison of cholesteryl ester synthesis by IC21 and HMD macrophages. Results indicated that all aspects of lipoprotein and cholesterol metabolism studied are similar for IC21 and mouse peritoneal macrophages. Thus IC21 macrophages are an acceptable model system for study of lipoprotein and cholesterol metabolism. (35 Refs)

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VIRUS ISOLATION/ STRUCTURE/ CHARACTERIZATION/ IDENTIFICATION

1. INVERTED REPEAT NUCLEOTIDE SEQUENCES IN THE GENOMES OF MAREK DISEASE VIRUS AND THE HERPESVIRUS OF THE TURKEY.

Cebrian J, Kaschka-Dierich C, Berthelot N, Sheldrick P
Institut de Recherches Scientifiques sur le Cancer, Centre National de la Recherche Scientifique, B.P. 8, 94802 Villejuif Cedex, France
Proc Natl Acad Sci USA; 79(2):555-559 1982

The DNAs of two herpesvirus, the oncogenic Marek disease virus and the serologically related herpesvirus of the turkey, were studied by electron microscopy. On the basis of fold-back molecules observed in single-stranded DNA from both viruses, structures have been derived for the overall nucleotide sequence arrangement in their genomes. Although differing in mol wt, the genomes of Marek disease virus and turkey herpes-

HE 20.3173/2:

virus are both constructed according to the same plan - two regions of unique nucleotide sequence, each enclosed by inverted repeat sequences. The genome structure of these viruses therefore closely resembles that of herpes simplex virus rather than the biologically more similar herpesvirus Epstein-Barr virus, H saimiri, and H ateles. (Author abstract) (46 Refs)

2. PHYSICAL MAP OF THE SHORT FOLDBACK SEQUENCES OF HERPES SIMPLEX VIRUS TYPE 1 DNA.

Miller RH, Russell RJ, Hyman RW
(c/o Hyman) Dept. Microbiology, Pennsylvania State Univ. Coll. Medicine, Hershey, PA, 17033
Virology; 117(1):70-80 1982

Short foldback sequences were found and mapped on cloned fragments of herpes simplex virus type 1 (HSV-1) DNA. Few foldbacks were within the long and short unique regions of HSV-1 DNA. However, the joint region contained numerous foldback sequences. (Author abstract) (24 Refs)

3. ANALYSIS OF POLYPEPTIDES OF THE TREE SHREW (TUPAIA) HERPESVIRUS BY GEL ELECTROPHORESIS.

Faissner A, Darai G, Flugel RM
Inst. Virus Res., German Cancer Res. Center, Im Neuenheimer Feld 280, 69 Heidelberg, W. Germany
J Gen Virol; 58(part1):139-148 1982

The virion polypeptide composition of three independently isolated tree shrew herpesviruses (THV) was analyzed by SDS-polyacrylamide slab gel electrophoresis and by a two-dimensional technique using isoelectric focusing. Two of the virus isolates analyzed were from malignant tumors; the other isolate (THV, strain 1) was from an apparently healthy animal. The polypeptide patterns of the three purified Tupaia herpesvirus isolates were remarkably similar, each consisting of at least 35 polypeptides ranging in mol wt from 12,000 to 230,000. While the majority of analogous polypeptides of the three viruses were of indistinguishable electrophoretic mobility, some (eg polypeptides of 82K to 86K) showed small differences in apparent mol wt which were characteristic of the virus strain. Comparative SDS-polyacrylamide gel electrophoresis made it possible to distinguish the Tupaia herpesvirus isolates from each other. At least five glycoproteins were found in purified THV virions. The two-dimensional electropherograms revealed at least 47 discernible protein spots, some of which were specific for a given THV isolate and which were detectable even in lysates of THV-infected cells. (Author abstract) (24 Refs)

VIRUS ASSOCIATED CELL TRANSFORMATION AND HOST ALTERATION

4. ADENOVIRUS-CELL INTERACTIONS EARLY AFTER INFECTION: IN VITRO CHARACTERISTICS AND TUMOURIGENICITY OF ADENOVIRUS TYPE 2-TRANSFORMED RAT LIVER EPITHELIAL CELLS.

Paraskeva C, Brown KW, Gallimore PH
Imperial Cancer Res. Fund, P. O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, England
J Gen Virol; 58(part1):73-81 1982

Cloned rat liver epithelial cells (clone C3) were semi-permissive for adenovirus type 2 (Ad-2) and non-permissive for adenovirus type 12 (Ad-12). Ad-2-infected C3 cells were shown to produce hexon and fiber protein, but at an moi of 20 a maximum virus yield of only 2.4 pfu per cell was obtained. Forty-eight hr after infection with Ad-12 'early' virus proteins (major species 8K and 60K), but no 'late' proteins (virus structural proteins) could be identified. Of six Ad-2-transformed epithelial lines isolated from clone C3 only one was tumorigenic in syngeneic rats, whereas all six transformants produced tumors in athymic nude mice. There was a remarkable variation in the morphology of the Ad-2-transformed liver cells, ranging from an epithelial morphology similar to C3 cells to cells with a distinct lymphoid morphology. The in vitro and in vivo behavior of the Ad-2-transformed clone C3 cells reported in this communication, taken together with our previous report on the characteristics of Ad-12-transformed C3 cells, clearly show that the differences observed between Ad-2-and Ad-12-transformed rat embryo cells were also observed in our studies using cloned rat liver epithelial cultures. Our findings clearly rule out the hypothesis that the heterogeneity of Ad-2 transformation events is the result of the transformation in vitro of different types of target cell. (Author abstract) (23 Refs)

5. PROTEINS CONTAINING ONLY HALF OF THE CODING INFORMATION OF EARLY REGION 1B OF ADENOVIRUS ARE FUNCTIONAL IN HUMAN CELLS TRANSFORMED WITH THE HERPES SIMPLEX VIRUS TYPE 1 THYMIDINE KINASE GENE AND ADENOVIRUS TYPE 2 DNA.

Klessig DF, Quinlan MP, Grodzicker T
Dept. Cellular, Viral and Molecular Biology, Univ. Utah, Salt Lake City, UT, 84132
J Virol; 41(2):423-434 1982

We introduced into tk- human 143 cells adenovirus Type 2 (Ad2) genes by transformation with a plasmid (p711) containing both Ad2 sequences and the herpes simplex virus Type 1 (HSV-1) tk gene. p711 contained approximately the left 8% of the Ad2 genome inserted in the HindIII site of pBR322, whereas the fragment of HSV-1 containing the tk gene was inserted in the BamHI site. Three tk+ cell lines were isolated after selection in HAT medium. The arrangement of viral sequences in the three transformants was analyzed by restriction endonuclease digestion and filter hybridization. All three lines contained a single insertion of Ad2 DNA which was present at approximately one copy per cell. The arrangement of Ad2 sequences in these lines was identical to that found in the linear p711 DNA used in the transformation. S1 analysis of the Ad2-specified RNA from two of these lines indicated that the early region 1a mRNA's were synthesized, though in lower amounts than found in lytic infections. These cell lines contained only the left half of early region 1b (4.6 to 11.2), which encoded the 5' portion of the 1b mRNA's. A complex pattern of 1b RNAs was made in these cell lines. Transcription of most of these RNAs began at or near the 1b promoter and proceeded through the 1b sequences into the flanking pBR322, HSV-1, or host sequences. Since many of the RNAs were terminated or spliced in the HSV-1 (anti-sense strand) or pBR322 sequences, new RNA processing sites must be used in the formation of these mRNA's. All three lines fully complemented the 1a deletion mutant Ad5 dl312. Surprisingly, these lines also permitted the growth of 1b deletion mutants (Ad5 dl313 and Ad5 dl434), although the complementation was not always complete. Presumably the new gene product(s) which contained only part of the 1b gene provided most of the essential function(s) required for viral multiplication. Alternatively, the 1b 19-kilodalton protein which was entirely encoded by the adenovirus sequences present in these cell lines was sufficient for viral growth even

in the absence of the 1b 55-kilodalton protein. (Author abstract) (50 Refs)

6. INTEGRATION AND EXPRESSION OF VIRAL DNA IN CELLS TRANSFORMED BY HOST RANGE MUTANTS OF ADENOVIRUS TYPE 5.

Ruben M, Bacchetti S, Graham FL
(c/o Graham) Dept. Pathology and Biology, McMaster Univ., Hamilton, Ontario L8N 3Z5, Canada
J Virol; 41(2):674-685 1982

Group I host range (hr) mutants of adenovirus Type 5 are unable to transform rat embryo or rat embryo brain cells but induce an abnormal transformation of baby rat kidney cells. We established several transformed rat kidney cell lines and characterized them with respect to the transformed phenotype and the structure of the integrated viral DNA. The hr mutant-transformed cells, unlike wild-type virus transformants, were fibroblastic rather than epithelial, failed to grow in soft agar, and were also less tumorigenic in nude mice. Studies on the structure of the integrated viral DNA sequences showed that hr-transformed cells always contained the left end of the adenovirus DNA, but the size of the integrated DNA fragment varied among different lines, and a high percentage of the lines contained the entire viral genome colinearly integrated. The patterns of integration were maintained after prolonged growth in culture and after subcloning. Attempts to rescue infectious virus from lines which contained the entire genome were unsuccessful. Using immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis, we analyzed the viral proteins expressed in hr-transformed cells. Results of these studies indicated that, like wild type-transformed cells, hr transformants expressed E1B proteins of mol wt 58,000 and 19,000. (Author abstract) (48 Refs)

7. SELECTIVE INHIBITION OF SV40-TRANSFORMED CELL GROWTH BY DIKETOPIPERAZINES.

Munekata M, Tamura G
Res. and Development Laboratories, Sapporo Breweries Ltd., Mita, Meguro-ku, Tokyo 153, Japan
Agric Biol Chem; 45(11):2613-2618 1981

Taxonomy of Streptomyces species strain ML1532, and isolation, characterization, and structures of the active substances produced by the strain were studied. Two diketopiperazines, cyclo(L-Val-L-Pro) and cyclo(L-Tyr-L-Pro) were isolated. Results showed that cytotoxicity of cyclo(L-Tyr-L-Pro) against SV40-transformed cells is slightly higher than that of cyclo(L-Val-L-Pro), and that the diketopiperazines may be metabolites of strain ML1532. (12 Refs)

8. CELL SURFACE BINDING AFFINITY OF SIMIAN VIRUS 40 T-ANTIGEN.

Lange-Mutschler J, Henning R
(c/o Henning) Dept. Biochemistry, Univ. Ulm, P.O. Box 4066, D-7900 Ulm, W. Germany
Virology; 117(1):173-185 1982

Simian virus 40-transformed cells are characterized by a virus-induced tumor transplantation antigen (SV40 TSTA) defined in vivo by the rejection of tumorigenic SV40-transformed cells in SV40-immunized mice and in vitro by SV40 tumor cell-specific cytotoxic T cells. Several experimental findings support the notion that SV40-infected and -transformed cells express SV40 large tumor antigen (T-Ag) or closely related antigens on the cell surface (surface T). In this report, evidence is presented for a cell surface binding affinity of SV40 T-Ag solubilized and extracted by disruption of SV40-transformed and SV40-infected cells in growth medium. Incubation of various transformed and nontransformed living monolayer cells in situ with these extracts led to a significant uptake of T-Ag to the cell surface (called 'externally bound T-Ag') up to 2-5x higher amounts in comparison to native surface T on SV40-transformed cells. This was demonstrated by the highly sensitive 125I-protein A assay using rabbit antisera directed against purified SV40 T-Ag. Serological analysis of T-Ag in cellular extracts and of externally bound T-Ag revealed no apparent differences suggesting the cell surface binding affinity as a new property of SV40 T-Ag. We interpret our results as an indication that this property enables purified T-Ag to initiate the cellular immune response necessary for the SV40-tumor rejection in mice. (Author abstract) (40 Refs)

9. SPECIFIC INTERACTION OF THE SV40 T ANTIGEN-CELLULAR P53 PROTEIN COMPLEX WITH SV40 DNA.

Reich NC, Levine AJ
(c/o Levine) Dept. Microbiology, State Univ. New York Stony Brook, Sch. Medicine, Stony Brook, NY, 11794
Virology; 117(1):286-290 1982

Three forms of the simian virus 40 (SV40) large tumor antigen (T antigen), free T antigen from infected monkey cells, T antigen-p53 complex from virus-infected mouse cells, and T antigen-p53 complex from SV40-transformed mouse cells, were examined for their abilities to bind specifically to SV40 DNA. Employing an immunoassay that detected T antigen and/or p53 protein bound to restriction fragments of viral DNA, it was shown that all three of these forms of SV40 T antigen bind preferentially to the restriction fragment of SV40 DNA containing the nucleotide sequence at the origin of viral DNA replication. Free p53 protein alone failed to bind to SV40 DNA in this assay. These results indicate that the SV40 T antigen-p53 protein complex displays the same SV40 DNA binding specificity as free T antigen obtained from a productive infection. (Author abstract) (24 Refs)

10. MOUSE MAMMARY TUMOUR VIRUS AND POLYOMA VIRUS INFORMATION IN MAMMARY TUMOURS OF ATHYMIC MICE INOCULATED WITH POLYOMA VIRUS.

Arya SK, Galarraga JJ
Lab. Tumor Cell Biology, NCI, NIH, Bethesda, MD, 20205
J Gen Virol; 58(part1):107-114 1982

Polyoma virus inoculation of athymic mice results in the development of mammary tumors with a much higher incidence than the development of salivary gland tumors, the latter being the most common for immunocompetent normal mice. The possibility existed that polyoma virus might act as a co-carcinogen in activating the expression of mouse mammary tumor virus (MMTV). Molecular hybridization studies, however, showed that the mammary tumor development was accompanied by neither the amplification of MMTV genomic sequences nor by their more extensive transcription. In contrast, tumor tissue contained about 60 to 100 copies of polyoma virus genome equivalents per cell and some of these sequences were apparently transcribed into RNA. While these results do not rule out the transient involvement of MMTV expression in mammary tumor development, it appeared that the mammary gland cells were directly transformed by polyoma virus. Apparently, polyoma virus displayed a tropism in athymic mice that was different from that in normal mice. (Author abstract) (30 Refs)

11. EXPRESSION OF TRANSFORMING REGION OF MOLONEY MURINE SARCOMA VIRUS IN ESCHERICHIA COLI AS A FUSION PROTEIN WITH SMALL TUMOR ANTIGEN OF POLYOMA VIRUS.

Donoghue DJ, Hunter T

Tumor Virology Lab., The Salk Inst., P.O. Box 85800, San Diego, CA, 92138

Proc Natl Acad Sci USA; 79(3):800-804 1982

Bacterial expression of the transforming region of Moloney murine sarcoma virus, designated mos, was obtained as a fusion protein with a portion of the small tumor antigen of polyoma virus. This was accomplished by fusing the entire mos open reading frame, encoding a 41,000-dalton protein, with a plasmid that expresses a beta-galactosidase-polyoma fusion protein under lac operon control. The resulting plasmid directed synthesis of the predicted polyoma antigen-sarcoma virus fusion protein of 59,000 daltons. This protein was immunoprecipitated by an antipolyoma tumor antigen antiserum that recognized polyoma determinants at the NH2 terminus of the hybrid protein. This protein was also immunoprecipitated by an antiserum directed against a synthetic peptide containing the 12 COOH-terminal amino acids encoded by the mos open reading frame. This work confirms the existence of a long open reading frame in the mos gene and resolves a discrepancy between different nucleotide sequences for its COOH-terminal coding region. (Author abstract) (32 Refs)

12. EXCISION OF POLYOMA VIRUS GENOMES FROM CHROMOSOMAL DNA BY HOMOLOGOUS RECOMBINATION.

Lania L, Boast S, Fried M

Istituto di Biologia Generale e Genetica, Universita di Napoli, Napoli, Italy

Nature; 295(5847):349-350 1982

Excision of polyoma virus genomes from chromosomal DNA was examined in five polyoma-transformed rat cell lines. One cell line contains multiple inserts of integrated viral sequences, free viral genomes and at least one complete early region containing an intact coding region for the polyoma large T antigen (T-Ag). Other four lines do not contain free viral forms or an intact coding region for the polyoma large T-Ag. All five lines contain intact coding regions for the small and middle T-Ag species. All five lines were fused either to themselves or to mouse cells and the fused cells were assayed for free viral forms (at varying times) or the production of infectious virus (after 5 days). In the self-fused cells, free viral forms were found only in the polyoma-rat cells; no virus was detected (less than 50 plaque-forming units/10(6) cells) in the self fusion of the polyoma-rat cells. The production of both free viral genomes and infectious virus was detected after fusion to mouse cell of polyoma-rat and two lines containing partial duplications of viral sequences. In the two lines that did not contain partial duplications of viral sequences, no free viral forms or infectious virus could be detected after fusion to mouse cells. In the fusions of 2b-C3 and 53-rat with mouse cells, the amount of viral DNA and infectious virus produced appears to be related to the amount of duplicated viral sequences present, suggesting that the excision may be a function of the target size for possible recombination events as has been observed in other systems. (32 Refs)

13. GASTRIN AND THE TRANSFORMING PROTEIN OF POLYOMA VIRUS HAVE EVOLVED FROM A COMMON ANCESTOR.

Baldwin GS

Melbourne Tumour Biology Unit, Melbourne, Australia

FEBS Lett; 137(1):1-5 1982

While gastrin and the transforming protein (middle T antigen) of polyoma virus appear to be superficially dissimilar molecules, a hitherto unreported structural relationship between the two proteins is presented, which supports the hypothesis that they have evolved from a common ancestor. (54 Refs)

14. PAPILLOMA VIRUS-INDUCED TUMORS CONTAIN A VIRUS-SPECIFIC TRANSCRIPT.

Freese UK, Schulte P, Pfister H

Institut fur Virologie, Zentrum fur Hygiene, Universitat Freiburg, Hermann-Herder-Strasse 11, D7800 Freiburg, W. Germany

Virology; 117(1):257-261 1982

Papillomavirus-specific transcription was investigated in the absence of virus p article production in BPV 1-induced hamster tumors. The analysis revealed a single virus-specific polyadenylated transcript of 1,300-base length, which was mapped on the genome of BPV 1. Hybridization with 32P-labeled probes confined the transcribed region to two HpaII fragments, which are adjacent to the BamHI cleavage site within the 1.4 x 10(6) dalton BamHI/EcoRI fragment. Related DNA sequences could be detected within the genomes of HPV 1 and HPV 4 using relaxed hybridization conditions. (Author abstract) (31 Refs)

15. VARIABLE-SIZED FREE EPISOMES OF SHOPE PAPILLOMA VIRUS DNA ARE PRESENT IN ALL NON-VIRUS-PRODUCING NEOPLASMS AND INTEGRATED EPISOMES ARE DETECTED IN SOME.

Wettstein FO, Stevens JG

Dept. Microbiology and Immunology, Sch. Medicine, Univ. California, Los Angeles, CA, 90024

Proc Natl Acad Sci USA; 79(3):790-794 1982

The state of rabbit (Shope) papilloma virus DNA in virus-induced nonproducing tumors on domestic rabbits was investigated. Virus-specific sequences were resolved into many distinct bands by one-dimensional agarose gel electrophoresis. Two-dimensional gel electrophoresis, CsCl/propidium iodide density equilibrium centrifugation, partial digestion with a restriction endonuclease, and SI nuclease digestion permitted us to identify the bands as free viral episomes representing circular molecules of increasing size. In some tumors (both papillomas and carcinomas), up to 25% of the virus-specific DNA was linear and comigrated with cellular DNA. Integration of at least some of these sequences was suggested by the detection of viral-cellular junction bands in one tumor after digestion of DNA with EcoRI and Sal I, enzymes that cut Shope DNA once. Finally, the physical states of viral DNA in papillomas and carcinomas were found to be similar, although free episomes were generally larger in carcinomas. (Author abstract) (22 Refs)

16. MALIGNANT TRANSFORMATION OF CELLS OF NEWBORN HAMSTERS BY HERPES SIMPLEX VIRUS TYPE 2.

Kitsak VIa
Central Inst. Advanced Training for Physicians, Moscow, USSR
Vopr Virusol; (6):750-753 1981

Malignant transformation induced by herpes simplex virus Type 2 (HSV-2) was studied in cultured fibroblasts from 3-5 day old hamsters. Infection of primary culture with UV-inactivated HSV-2 caused morphological transformation of cells; first signs of morphological transformation were detected 14-25 days after infection. Characteristic features of morphological transformation were the loss of contact inhibition, multilayer growth, and progressive replacement of epithelioid cells by fibroblastlike cells. Immunofluorescent analysis showed the presence of virus-specific antigen in 5-30% of transformed cells, but transformed cells did not produce the infectious virus. Syngeneic newborn hamsters inoculated sc with transformed cells developed fibrosarcomas. Duration of the latent period was cor related with the number of passages in vitro (from 12 wk after 27 passages to 4 wk after 50 passages). (11 Refs) (In RUS)

17. CORRELATION OF THE VIRUS SEQUENCE CONTENT AND BIOLOGICAL PROPERTIES OF CELLS CARRYING THE HERPES SIMPLEX VIRUS TYPE 2 THYMIDINE KINASE GENE.

Minson A, Bell SE, Bastow K
Dept. Pharmacology, Univ. North Carolina, Chapel Hill, NC, 27514
J Gen Virol; 58(part1):127-138 1982

Seven cell lines, transformed to a thymidine kinase-positive phenotype with herpes simplex virus type 2 (HSV-2) DNA fragments, have been examined to determine their virus-specific DNA sequence content. The results are consistent with the reported map position of the HSV-2 thymidine kinase gene. Different cell lines contained different amounts of non-selected virus-specific sequences and this correlated with the ability of some cell lines to compensate for the deficiencies in some temperature-sensitive (ts) mutants of HSV-1 and HSV-2. The cell lines were also examined for their ability to synthesize elevated levels of thymidine kinase in response to infection with a thymidine kinase-negative virus mutant. Of the seven cell lines, two failed to respond to infection in this way, and these cell lines lacked sequences on the upstream side of the kinase gene which were present in the remaining cell lines. (Author abstract) (41 Refs)

18. UPDATE ON THE RELATION OF HERPESVIRUS HOMINIS TYPE II TO CARCINOMA OF THE CERVIX.

Fish EN, Tobin SM, Cooter NB, Papsin FR
Div. Infectious Diseases, Hosp. for Sick Children, 555 Univ. Ave., Toronto, Ontario M5G 1X8, Canada
Obstet Gynecol; 59(2):220-224 1982

Animal studies are described that investigate which cofactors influence the progression of herpesvirus hominis Type II (HVH-II)-induced atypia of the cervical epithelium to malignancy. Results indicate that neither chemical nor physical factors markedly affect typical development. However, sperm factors are implicated in influencing this atypical development. These data are considered in relation to the etiologic role of HVH-II in carcinoma of the cervix. (Author abstract) (12 Refs)

19. LINKAGE MAP OF COTRANSFECTED VIRAL DNA SEQUENCES IN A MOUSE 3T3 CELL LINE.

van den Berg FM, Walboomers JM
(c/o Walboomers) Lab. Pathological Anatomy, Section Experimental Oncology, Wilhelmina Gasthuis, Eerste Helmersstraat 104, 1054 EG Amsterdam, The Netherlands
Intervirology; 16(3):184-190 1981

A physical map was constructed of a cotransformed 3T3 thymidine kinase (tk) negative cell line revealing a close linkage of the selectable herpes simplex virus Type 2 tk gene and cotransfected simian virus 40 DNA sequences in high-mol-wt cellular DNA. (Author abstract) (12 Refs)

20. A 53 KILODALTON PROTEIN COMMON TO CHEMICALLY AND VIRALLY TRANSFORMED CELLS SHOWS EXTENSIVE SEQUENCE SIMILARITIES BETWEEN SPECIES.

Jornvall H, Luka J, Klein G, Appella E
Dept. Chemistry I, Karolinska Institutet, S-104 01 Stockholm, Sweden
Proc Natl Acad Sci USA; 79(2):287-291 1982

A heat-stable DNA-binding protein with subunits of about 53 kilodaltons (kDal) was purified from two virally transformed human cell lines (Epstein-Barr virus-positive Raji and Namalwa) and two mouse tumor cell lines (methylcholanthrene-induced Meth A sarcoma and TA3 mammary carcinoma). All four 53-kDal proteins showed closely related total amino acid compositions, similar peptide maps, and identical NH2-terminal amino acid sequences for 20 residues. These 53-kDal proteins are therefore evolutionarily highly conserved, independent of whether they originate from virally or chemically transformed cells. The NH2-terminal sequence and the protein chain as a whole are not hydrophobic; however, some unexpected residue distributions were observed. Comparisons with other proteins reveal no clear sequence similarity with known tumor antigen structures, homologous immunoglobulins, or some other proteins of known sequence. Epstein-Barr virus-determined nuclear antigen also appears to have a different NH2-terminal sequence. Thus, the results show that the 53-kDal proteins represent a unique protein type with little species variation; this finding suggests that these proteins must perform an important common function in different transformation systems. (Author abstract) (38 Refs)

21. ALKALINE PHOSPHODIESTERASE I AND ALKALINE PHOSPHATASE I IN PLASMA MEMBRANES OF HERPES SIMPLEX VIRUS TYPE 1 TRANSFORMED HAMSTER CELLS.

Schneider D, Brauer D, Falke D
(c/o Falke) Inst. Medical Microbiology, Div. Experimental Virology, Johannes Gutenberg-Univ., Hochhaus Augustusplatz, D-6500 Mainz, W. Germany
Biochim Biophys Acta; 685(1):94-104 1982

Plasma membrane extracts from Herpes simplex virus Type 1 transformed hamster embryo fibroblasts were chromatographed on Lens culinaris lectin coupled to Sepharose (LcH-Sepharose) and analyzed by dodecyl sulfate polyacrylamide gel electrophoresis. Coomassie blue-staining revealed two major protein bands with apparent mol wt of 125,000 and of about 75,000-90,000. In plasma membranes isolated from these tumor cells prior labeled with [3H]fucose or [3H]glucosamine these bands contained the highest amounts of incorporated radioactivity. Separation by LcH-Sepharose-affinity chromatography as well as metabolic labeling clearly demonstrates their glycoprotein character. The 125,000 protein coincides with alkaline

phosphodiesterase I activity with a Km of 6.10(-4) M for TMP p-nitrophenyl ester and is competitively inhibited by UDP-N-acetylglucosamine. This enzymatic activity is also present in normal hamster embryo fibroblasts. Gel electrophoresis of the Lens culinaris lectin-binding glycoproteins from plasma m embranes of normal hamster embryo fibroblasts additionally revealed a strong alkaline phosphatase activity represented by an apparent mol wt of 150,000, while HSV1 hamster tumor cells contain only a very weak activity of this enzyme activity. HSV-lytically infected cells, however, have unchanged levels of alkaline phosphatase activity, whereas alkaline phosphodiesterase activity increases slighily. (Author abstract) (36 Refs)

22. POTENCY RANKING OF CHEMICALS BASED ON ENHANCEMENT OF VIRAL TRANSFORMATION.

Casto BC
Northrop Services, Inc., Research Triangle Park, NC
Potency Ranking of Chemicals Based on Enhancement of Viral Transformation. Available from National Technical Information Service, Springfield, VA, as PB81-210080:, 41 pp., 1981.

Treating primary hamster embryo cells with various classes of chemical carcinogens and mutagens leads to enhancement of transformation by simian adenovirus SA7. It appears that carcinogenic chemicals render the individual cells more sensitive to viral transformation, thus increasing the total number of cells integrating SA7 DNA. Enhancement of viral transformation appears to be a sensitive indicator for chemical agents with the potential to damage cell DNA by either direct or indirect means and thus may be useful as a screening tool to detect these chemicals in the environment. This report summarizes and compares the results for 134 chemicals (both carcinogenic and noncarcinogenic) assayed for enhancement of SA7 transformation, chemical transformation, and induction of DNA strand breaks and DNA repair synthesis. In addition, these chemicals are ranked by lowest effective concentration in the assay for enhancement of viral transformation. (Author abstract)

VIRUS REPLICATION AND EXPRESSION

23. ADENOVIRAL HETEROGENEOUS NUCLEAR RNA IS ASSOCIATED WITH THE HOST NUCLEAR MATRIX DURING SPLICING.

Mariman EC, Van Eekelen CA, Reinders RJ, Berns AJ, Van Venrooij WJ
Dept. Biochemistry, Univ. Nijmegen, Geert Grooteplein Noord 21, 6525 EZ Nijmegen, The Netherlands
J Mol Biol; 154(1):103-119 1982

After infection of HeLa cells with adenovirus type 2, virus-specific heterogeneous nuclear RNA is quantitatively associated with a higher ordered structure, the nuclear matrix. Anslysis of this matrix-associated RNA by S1 nuclease mapping showed that precursors as well as processed messenger RNAs from the late region L4 were present. By irradiation of intact cells with ultraviolet light, proteins tightly associated with heterogeneous nuclear RNA can be induced to cross-link with the RNA. Characterization of the cross-linked RNA-protein complexes showed that all viral polyadenylated RNAs (precursors, products and processing intermediates) could be cross-linked to two host proteins, earlier found to be involved in the association of host-specific heterogenous nuclear RNA to the nuclear matrix. Our results thus further support the concept that the nuclear matrix may function in the localization and the structural organization of (viral) heterogeneous nuclear RNA during its processing. (Author abstract) (31 Refs)

24. R-LOOP MAPPING OF CALF-THYMUS RNA POLYMERASE II. TRANSCRIPTS IN VITRO ON RESTRICTION FRAGMENTS OF ADENOVIRUS 2 DNA.

Spindler E, Stein H
Max-Planck-Institut fur Biologie, Abteilung Beermann, Spemannstrasse 34, D-7400 Tubingen, W. Germany
Eur J Biochem; 121(2):289-294 1982

Nascent RNA, synthesized by calf thymus RNA polymerase II on the restriction endonuclease EcoRI fragment A and BamHI fragment B of adenovirus 2 DNA, was rehybridized to the template strand under conditions allowing transcription R-loop formation. Hybrids, visualized by electron microscopy, were found in looped and in branched configurations, the former being abundant, with an average loop number of roughly four per EcoRI fragment A and three per BamHI fragment B. Frequency distributions of transcription R-loops, synthesized on identical restriction fragments for different periods of time, showed similar patterns, thus pointing to a nonrandom type of transcription. A comparison of the frequency distribution of R-loops seen on the two overlapping restriction fragments reveals reasonable coincidence in the loop pattern in one out of the four possible fragment orientations, permitting correlation of the transcription R-loop pattern with the physical map of the adenovirus 2 DNA. Comparison of start sites tentatively mapped in vitro with the known promoters of RNA polymerase II in vivo suggests that a major transcription site in vitro is located in close proximity to, or identical with, two promoters in vivo at 16.1 and 16.5 map units. (Author abstract) (22 Refs)

25. STRUCTURE OF ADENOVIRUS CHROMATIN.

Mirza MA, Weber J
(c/o Weber) Departement de Microbiologie, Centre Hospitalier Universitaire, Universite de Sherbrooke, Sherbrooke, P.Q. J1H 5N4, Canada
Biochim Biophys Acta; 696(1):76-86 1982

The structure of adenovirus Type 2 chromatin isolated from wild-type and ts1 virions was investigated by micrococcal nuclease digestion and electron microscopy. Partial digestion of wild-type and ts1 chromatin with micrococcal nuclease generated a multimeric DNA smear devoid of the 200 basepair nucleosome repeating pattern characteristic of cellular chromatin. However, 11 S monomer cores of 150 basepairs were detectable. The chromatin of ts1 (39 C) was more resistant to digestion by micrococcal nuclease. Two-dimensional electrophoresis of the monomer core showed that wild-type core contained protein VII while ts1 (39 C) core contained PVI and PVII. Protein V appears to be located on the variable-length intermonomer region. Crosslinking studies suggest that proteins PVII and VII exist in dimeric form wi thin the monomer core. Electron microscopy revealed a 5.5-fold-condensed 2-micron-long beaded structure with about 200 monomer particles spaced irregularly. Based on these observations, a model for adenovirus prochromatin and chromatin is proposed that differs in important aspects from the model proposed previously. (Author abstract) (26 Refs)

26. RESOLVING THE FUNCTIONS OF OVERLAPPING VIRAL GENES BY SITE-SPECIFIC MUTAGENESIS AT A MRNA SPLICE SITE.

Montell C, Fisher EF, Caruthers MH, Berk AJ
Dept. Microbiology, Univ. California, Los Angeles, CA, 90024
Nature; 295(5848):380-384 1982

Early region IA of human adenoviruses encodes a function required for normal induction of early viral genes and virus-induced cell transformation. The region is expressed at early times as two overlapping spliced mRNAs, 12S and 13S, which encode closely related proteins. To distinguish between the functions of these proteins, a single T to G transversion was constructed which prevents splicing of the 12S mRNA. This transversion, in the second base of the 12S mRNA intron, does not alter the protein encoded by the 13 mRNA due to degeneracy in the genetic code. Studies with this mutant demonstrated that only the 13S mRNA encodes the regulatory protein required for normal early gene expression. (Author abstract) (47 Refs)

27. MOLECULAR INTERACTION BETWEEN SIMIAN VIRUS 40 DNA AND ESCHERICHIA COLI RNA POLYMERASE. MAPPING OF THE INITIATION SITES ON SUPERCOILED AND LINEAR DNA.

Lavialle C, Reuveni Y, Thoren M, Salzman NP
Lab. Biology Viruses, Natl. Inst. Allergy and Infectious Diseases, NIH, Bethesda, MD, 20205
J Biol Chem; 257(3):1549-1557 1982

A detailed map of the initiation sites recognized by the Escherichia coli RNA polymerase (holoenzyme) on simian virus 40 (SV40) DNA has been constructed. An approach is described in order to compare the sites recognized on supercoiled as well as relaxed linear DNA. For localization of the initiation sites on supercoiled DNA, RNA polymerase-SV40 DNA Form I binary complexes are stabilized by incubation with three ribonucleoside triphosphates. The stable initiation complexes are cleaved with single cut restriction endonucleases in order to generate termination sites at precisely defined locations. Elongation of the RNA chains up to the cleavage site produces a defined set of discrete RNA species, whose size can be determined with accuracy by agarose gel electrophoresis following glyoxal denaturation. Each transcript is oriented by following two different experimental approaches. (1) The DNA of an SV40 deletion mutant is used as a template in comparison with that of wild type SV40; and (2) the RNAs synthesized after cleavage with different single cut restriction endonucleases are compared. Determination of the length and polarity of these RNAs allows us to accurately position the initiation sites on the physical map of the SV40 genome. A similar analysis was conducted for the mapping of the initiation sites recognized on precut linear SV40 DNA. Comparison of the two templates (supercoiled and linear) reveals that modification of the DNA conformation affects selection by the enzyme of certain promoters. Although most of the promoters are common to both templates, four are specifically recognized on superhelical DNA, while two others are used exclusively on linear SV40 DNA. (Author abstract) (47 Refs)

28. ELECTRON MICROSCOPE MAPPING OF RNA POLYMERASE BINDING SITES ON SV40 DNA.

Vogel F, Scherneck S
Zentralinstitut fur Molekularbiologie, Akademie der Wissenschaften der DDR, DDR-1115 Berlin-Buch, E. Germany
Gene; 16(1-3):331-334 1981

RNA polymerase from Escherichia coli was bound to the genome of simian virus 40 (SV40) after cleavage of the DNA with EcoRI or HpaII restriction enzymes. The enzyme DNA complexes were studied by electron microscopy. Nine binding sites were identified, including five in the 'early' region and four minor sites in the 'late' region of the SV40 DNA. Results suggest that the early region is a strongly conserved part of the SV40 genome. (17 Refs)

29. RELATIONSHIP OF OLIGOMERIZATION TO ENZYMATIC AND DNA-BINDING PROPERTIES OF THE SV40 LARGE T ANTIGEN.

Bradley MK, Griffin JD, Livingston DM
Sidney Farber Cancer Inst., Boston, MA, 02115
Cell; 28(1):125-134 1982

Crude and highly purified SV40 large T antigen has been found to exist in forms of various sizes. Immunoreactive structures of 5.5S (80-85 kd), 7S (approx 150 kd) and 15.5S (325-340 kd) have been identified by zonal sedimentation and gel filtration. They appear to correspond to monomeric, dimeric and tetrameric species of T, respectively, and are free of detectable 55 kd NVT by specific immunoprecipitation analyses. While highly purified monomer appears relatively inactive in SV40 DNA-binding and ATPase asays, both the dimer and tetramer display these activities. By contrast, all three comigrate with casein kinase activity. These data suggest that the protein can exist as a monomer and in various homoaggregated forms. In addition, it appears that it must aggregate to be an active DNA-binding element and an ATPase. (Author abstract) (46 Refs)

30. BIOLOGY OF SIMIAN VIRUS 40 (SV40) TRANSPLANTATION ANTIGEN (TRAG). VIII. RETENTION OF SV40 TRAG SITES ON PURIFIED SV40 LARGE T ANTIGEN FOLLOWING DENATURATION WITH SODIUM DODECYL SULFATE.

Flyer DC, Tevethia SS
(c/o Tevethia) Dept. Microbiology, Coll. Medicine, Pennsylvanian State Univ., Hershey, PA, 17033
Virology; 117(1):267-270 1982

Simian virus 40 (SV40) large tumor (T) antigen was isolated from SV40-infected permissive and -transformed cells by immunoprecipitation with anti-T sera and purified by electrophoresis on solubilizable SDS-polyacrylamide gels. This method proved to be a rapid and efficient procedure for purifying T antigen as 74% of the immunoprecipitated T antigen was subsequently recovered. The purified, SDS-denatured large T antigen retained its ability to react with anti-T antibody and to immunize mice against a challenge of syngeneic SV40 tumor cells, indicating that the antigenic sites present on the large T antigen molecule that induce a tumor rejection response in vivo are resistant to denaturation by SDS. (Author abstract) (25 Refs)

31. COMPARISON OF THE POLYOMA VIRUS EARLY AND LATE PROMOTERS BY TRANSCRIPTION IN VIVO.

Jat P, Cowie A, Kamen R
Transcription Lab., Imperial Cancer Res. Fund, P.O. Box 123, Lincoln's Inn Fields, London, England
Nucleic Acids Res; 10(3):871-887 1982

Polyoma virus DNA was transcribed in the HeLa whole cell extract in vitro system. Early region transcripts with the same 5'-ends as in vivo mRNAs, located 31 +-2 bp from 'TATA'-boxes, were synthesized by RNA polymerase II. Sequences sufficient for efficient expression of the early promoter were present in a substitution mutant lacking viral DNA from a position 55bp before the principal cap sites. Late region transcripts were synthesized inefficiently. Only one (at nt5129 +-2) of the many late mRNA cap sites functioned as an in vitro initiation point. This was the one 5'-end located 31 +-2 bp from a sequence resembling the 'TATA' consensus. The proportion of late to early region RNA polymerase II transcripts decreased dramatically at suboptimal template concentrations. An hypothesis to explain the regulation of late gene expression in vivo based on these results is proposed. Although linear templates were transcribed only by RNA polymerase II, transcripts with the same sense as late mRNAs and 5'-ends at nt5076 +-2 were produced from superhelical templates by an alpha-amanitin resistant enzyme. (Author abstract) (52 Refs)

32. A COMPARISON OF THE PHAGE T4 GENE 32 PROTEIN AND ESCHERICHIA COLI RNA POLYMERASE BINDING SITES ON HAMSTER PAPOVAVIRUS DNA.

Vogel F, Scherneck S
Zentralinstitut fur Molekularbiologie, Akademie der Wissenschaften der DDR, Lindenberger Weg 70, DDR-1115 Berlin-Buch, E. Germany
Biochim Biophys Acta; 696(1):102-106 1982

Phage T4 gene 32 protein and Escherichia coli RNA polymerase were bound to hamster papovavirus DNA. The binding regions were identified by electron microscopy employing a protein-free spreading technique. After gene 32 protein treatment four denaturation regions could be mapped, at 0.04-0.12, 0.30-0.36, 0.50-0.60 and 0.75-0.90 DNA map units, respectively, using the unique BamHI cleavage site as zero point. Eight RNA polymerase binding sites can be found which are localized at positions 0.05; 0.11; 0.18; 0.31; 0.57; 0.66; 0.76 and 0.82. A comparison of the RNA polymerase binding sites with the gene 32 protein denaturation pattern reveals a correspondence of six of eight polymerase binding sites with (A + T)-rich regions within the hamster papovavirus genome. (Author abstract) (20 Refs)

33. DNA-POLYMERASE INDUCED BY HERPESVIRUS PAPIO (HVP) IN CELLS OF LYMPHOBLASTOID CULTURES DERIVED FROM LYMPHOMATOUS BABOONS. - REPORT V.

Djachenko AG, Lapin BA
(c/o Lapin) Inst. Experimental Pathology and Therapy, USSR Acad. Medical Sciences, Gora Trapetsiya, P. B. 66, Sukhumi, USSR
Exp Pathol; 20(1):51-57 1981

A new DNA-polymerase was found in the cells of suspension lymphoblastoid cultures which produce lymphotropic baboon herpesvirus (HVP). This enzyme was isolated in a partially purified form. Some of its properties vary from those of other cellular DNA-polymerases. HVP-induced DNA-polymerase has a molecular weight of 160,000 and sedimentation coefficent of about 8 S. The enzyme is resistant to high salt concentration and N-ethylmaleimide, but it is very sensitive to phosphonoacetate. It effectively copies 'activated' DNA and synthetic deoxyribohomopolymers. Attempts to reveal the DNA-polymerase activity in HVP virions were unsuccessful. (Author abstract) (15 Refs)

34. A 3' CO-TERMINUS OF TWO EARLY HERPES SIMPLEX VIRUS TYPE 1 MRNAS.

McLauchlan J, Clements JB
Inst. Virology, Univ. Glasgow, Glasgow G11 5JR, Scotland
Nucleic Acids Res; 10(2):501-512 1982

A 3' co-terminus of two early herpes simplex virus type 1 mRNAs has been identified using the nuclease-S1 mapping procedure with cloned virus DNA probes. These mRNAs (5.0 kb and 1.2 kb), located within the genome region 0.56-0.60, are unspliced and are transcribed rightwards on the prototype genome orientation. The position of their 3' ends has been located on the virus DNA sequence and lies downstream from the polyadenylation signal 5'-AATAAA-3'. This hexanucleotide sequence also was present in the complementary DNA strand and was shown to be the polyadenylation signal for a leftwards-transcribed late mRNA. The abundance within the cytoplasm of the 5.0 kb and 1.2 kb mRNAs was investigated. Results indicated that these mRNAs were regulated in concert. It is suggested that sequences at the 3' co-terminus may be involved in their regulation. (Author abstract) (25 Refs)

35. ENZYMATIC DEOXYGLUCOSYLATION OF CERAMIDES BY MICROSOMES OF BHK-21 CELLS. THE EFFECT OF DEOXYGLUCOSE TREATMENT AND HERPESVIRUS INFECTION.

Suzuki Y, Blough HA
(c/o Blough) Dept. Ophthalmology, Univ. Pennsylvania Sch. Medicine, Scheie Eye Inst., Philadelphia, PA, 19104
Biochim Biophys Acta; 710(2):221-229 1982

The microsomal fractions of cultured hamster fibroblasts (BHK-21 cells) catalyze the incorporation of glucose from UDPglucose or of deoxyglucose from UDPdeoxyglucose into a reaction mixture with liposomes consisting of ceramide and phosphatidylcholine. The microsomal fractions also catalyze the transfer of glucose from UDPglucose to endogenous acceptors. The specific activity of ceramide deoxyglucoside or ceramide glucoside formation was significantly higher when microsomal preparations obtained from deoxyglucose-treated or herpesvirus-infected BHK-21 cells were used as the glucosyltransferase source. Deoxyglucose was incorporated from UDP-deoxyglucose into hydroxy- and nonhydroxy-fatty acid-containing ceramides at approx the same rate. Competitive inhibition of deoxyglucosylation of ceramides by UDPglucose suggests that both reactions were catalyzed by the same enzyme, viz UDPglucose:ceramide glucosyltransferase. This inhibition of glycosphingolipid synthesis may account, in part, for the inhibitory effect of deoxyglucose on lipid-containing viruses. (Author abstract) (22 Refs)

36. DNA SEQUENCE OF AN IMMEDIATE-EARLY GENE (IE MRNA-5) OF HERPES SIMPLEX VIRUS TYPE I.

Watson RJ, Vande Woude GF
Lab. Molecular Oncology, NCI, NIH, Bethesda, MD, 20205
Nucleic Acids Res; 10(3):979-991 1982

We describe a 2560 base pair herpes simplex virus Type 1 (HSV-1) DNA sequence containing the entire immediate-early mRNA-5 (IEmRNA-5) gene. The 3' and 5' termini of IEmRNA-5 were mapped within this DNA sequence by

single-strand specific endonuclease protection experiments. The IEmRNA-5 gene contains DNA sequences from both the unique (Us) and reiterated (TRs/IRs) regions of the HSV-1 DNA short component and is interrupted by a single intron mapping in TRs/IRs. A search of the transcribed DNA sequence revealed no initiator codon within TRs/IRs. The first ATG was located six bases into Us sequences and this reading frame was open for a further 87 codons. A second longer open reading frame (316 codons) was also observed in the 3' transcribed region. The oligonucleotide sequences adjacent to the IEmRNA-5 termini are discussed in relation to those of the HSV-1 thymidine kinase gene and other genes transcribed by RNA polymerase II. (Author abstract) (35 Refs)

37. MICROSCOPIC EXAMINATION OF THE INTRACELLULAR FATE OF INFECTIOUS HERPES SIMPLEX VIRUS DNA.

Marciano-Cabral F, Cabral GA, Taylor MM, Patterson M, Farber FE
Dept. Microbiology, Medical Coll. Virginia, VCU, Box 678 - MCV Station, Richmond, VA, 23298
Intervirology; 16(2):95-105 1981

The intracellular fate of 3H-labeled exogenous herpes simplex virus Type 2 DNA following the exposure of recipient cells to selected DNA facilitators and the ultrastructural changes which ensued in transfected cultures were examined. In most cases, electron microscopic radioautography demonstrated that exogenous DNA reached the nucleus within 1 hr postinoculation. At later time periods, enlargement of nuclei and margination of chromatin were noted. Cells treated with polyornithine differed significantly from those treated with other facilitators in that large cytoplasmic accumulations of labeled material associated with electron-dense, membrane-bound bodies persisted after 1 hr postinoculation. In contrast, labeled DNA released by intact virions was detected in the nucleus as early as 20 min postinfection (pi), and progeny virus was evident by 8 hr pi. These results suggest that the efficiency of each facilitator is related to the rapidity with which exogenous DNA penetrates the recipient cell, traverses the cytoplasm, and the amount of DNA which is finally associated with the nuclear compartment. (Author abstract) (15 Refs)

38. INCORPORATION OF E-5-(2-HALOVINYL)-2'-DEOXYURIDINES INTO DEOXYRIBONUCLEIC ACIDS OF HERPES SIMPLEX VIRUS TYPE 1-INFECTED CELLS.

Allaudeen HS, Chen MS, Lee JJ, De Clercq E, Prusoff WH
(c/o Prusoff) Dept. Pharmacology, Yale Univ. Sch. Medicine, New Haven, CT, 06510
J Biol Chem; 257(2):603-606 1982

E-5-(2-Bromovinyl)-2'-deoxyuridine (BrvdUrd) and E-5-(2-iodovinyl)-2'-deoxyuridine (IvdUrd) are among the most potent and selective inhibitors of herpes simplex virus type 1 (HSV-1) replication. To elucidate the site of inhibition, we examined whether the halovinyl analogs are incorporated into DNA using two approaches: (i) In assays with purified DNA polymerases omitting dTTP from the reaction system, addition of either BrvdUTP or IvdUTP increased the polymerization reaction, indicating that these two analog triphosphates can be alternate substrates. (ii) When HSV-1-infected Vero cells were grown in the presence of either BrvdUrd or IvdUrd, there was an increase in the density of both the viral and cellular DNA. The viral DNA had 40% of its thymidine moiety substituted by IvdUrd when the concentration of [125I]IvdUrd was 24 uM (in the absence of added thymidine). At 30 uM BrvdUrd and 1 uM [2-14C]thymidine, the viral DNA had only 11% of its thymidine moiety substituted by BrvdUrd, presumably because of the presence of added thymidine. Following digestion of [125I]IvdUrd-substituted DNA with DNase 1, venom phosphodiesterase, and alkaline phosphatase, the radioactivity co-migrated with nonradioactive IvdUrd in thin layer chromatography. Under similar conditions, no detectable incorporation of either [125I]IvdUrd or BrvdUrd into mock-infected Vero cell DNA was observed. Thus, IvdUrd and BrvdUrd are incorporated into DNA of HSV-1 infected cells but not into DNA of uninfected cells. (Author abstract) (22 Refs)

IMMUNOLOGICAL STUDIES

39. ADENOVIRUS TYPE 12 T ANTIGEN-RELATED SURFACE ANTIGEN DETECTED BY IMMUNOFLUORESCENCE ON THE MEMBRANE OF TRANSFORMED AND INFECTED CELLS.

Vasconcelos-Costa J, Ribeiro G
Unit Virology, Gulbenkian Inst. Science, Apartado 14, P-2781 Oeiras Codex, Portugal
Virology; 117(1):135-145 1982

THA cells, derived from a hamster tumor induced by adenovirus type 12 (Ad12), we re analyzed by immunofluorescence for the presence of virus-specific surface antigens. S antigen is a surface antigen detected on suspended living cells by a serum collected from hamsters immunized with Ad12. Besides S antigen, a new surface antigen (surface-T) was observed in formaldehyde-fixed cells treated with anti-T serum. Anti-T serum is not reactive against S antigen in living cells and anti-S serum did not detect surface-T antigen. The membrane location of surface-T antigen was confirmed with a Staphylococcus aureus binding assay. Surface-T antigen seems specific for Ad12, as it is not observed in hamster embryo cells, and is not recognized by antisera against SV40-or polyoma-T antigens nor by antiserum against hamster embryonic antigens. Surface-T antigen is also seen in infected KB cells but not in abortively infected RK13 cells. Anti-P serum, raised against an extract of infected RK13 cells, failed to detect surface-T antigen. Experiments with adsorbed anti-T and anti-S sera confirmed the specificity of surface-T antigen and its distinctiveness from S antigen. (Author abstract) (22 Refs)

40. UNIFORM NOMENCLATURE FOR MONOCLONAL ANTIBODIES DIRECTED AGAINST VIRUS-CODED PROTEINS OF SIMIAN VIRUS 40 AND POLYOMA VIRUS.

Crawford L, Harlow E
Molecular Virology Lab., Imperial Cancer Res. Fund, Lincoln's Inn Fields, London WC2A 3PX, England
J Virol; 41(2):709 1982

A uniform nomenclature has been agreed upon for monoclonal antibodies directed against virus-coded proteins of simian virus 40 and polyoma virus. Blocks of numbers from PAb1 to PAb999 have been allocated to workers involved in the isolation of monoclonal antibodies of this type. The correspondence between PAb numbers and previously used names is given. (Author abstract) (6 Refs)

41. KINETICS OF THE IMMUNE RESPONSE OF TUMOR-BEARING HAMSTERS TO TWO SIMIAN VIRUS 40 CODED NON-STRUCTURAL POLYPEPTIDES PRESENT IN SIMIAN VIRUS 40 TUMOR CELLS.

Ransom JH, Thompson LL, Tevethia SS
(c/o Tevethia) Dept. Microbiology and Specialized Cancer Center, Pennsylvania State Univ. Coll. Medicine, Hershey, PA, 17033
Int J Cancer; 29(2):217-222 1982

The kinetics of the immune response of hamsters transplanted with virus-free SV40 tumor cells (TSV5) to two SV40-coded non-structural proteins of 94,000 daltons (T antigen) and 17,000 daltons (T antigen) was studied in order to determine if the variation in the immune reactivity of serum from tumor-bearing animals towards these proteins can be explained on the basis of differential immune response during various stages of tumor growth. The results demonstrate that individual animals vary in their capacity to respond immunologically to T and t antigens in SV40-transformed cells and that, further, the immune response to T and t antigens is influenced by the stage of tumor development. (Author abstract) (31 Refs)

42. THE BINDING AFFINITY OF POLYOMA-TUMOR SPECIFIC ANTIGEN (TSTA) TO RECEPTORS OF LYMPHOCYTE POPULATIONS.

Ochalek T, Porwit-Bobr Z, Radzioch D
Dept. Microbiology and Immunology, Inst. Molecular Biology, Jagiellonian Univ., Krakow, Grodzka 53, Poland
Folia Histochem Cytochem (Krakow); 19(4):189-196 1981

The incorporation of 3H-thymidine was used as a means to determine the stimulation of thymus and lymph node cell populations by TSTA obtained and purified from the surface of polyoma cells in a conditioned medium with T cell growth factor. The specificity of TSTA was demonstrated after the immunization of CBA mice in adoptive transfer of lymph node cells followed by challenge with polyoma cells. The binding affinity of TSTA to receptors of lymph node cells and thymocytes from CBA mice was tested by radiolabeling. Differences in binding affinity estimated on the basis of equilibrium constant (K) and maximal binding capacity (n) were found between thymus and lymph node cell populations but not between lymph node cell populations themselves. The lack of change in the binding affinity of 125I-TSTA to receptors of lymph node cells in various experimental mice was also discussed. (Author abstract) (20 Refs)

43. SPECIFIC THYMUS-DERIVED (T) CELL RECOGNITION OF PAPOVA VIRUS-TRANSFORMED CELLS.

Greene MI, Perry LL, Kinney-Thomas E, Benjamin TL
Dept. Pathology, Harvard Medical Sch., 25 Shattuck St., Boston, MA, 02115
J Immunol; 128(2):732-736 1982

As a first step toward identifying papova virus-specific transplantation antigens and characterizing the immune response to these antigens, a series of congenic B10 mouse lines transformed by polyoma virus and SV40 has been obtained. B10.D2 (H-2d) mice immunized with x-irradiated polyoma-transformed B10.D2 cells (PyB10.D2) contained within their spleens a population of T cells that upon restimulation in vitro preferentially lyse 51Cr-labeled PyB10.D2 compared with other allogeneic polyoma-transformed target cells or SV40-transformed B10.D2 cells. Twenty hr after acute infection with polyoma virus, SV10.D2 cells express cell surface structures recognizable by polyoma-specific CTL. This CTL activity is therefore both polyoma specific and H-2 restricted, indicating associative recognition of H-2 molecules along with the virus-specific antigen. (Author abstract) (39 Refs)

44. B LYMPHOBLAST ANTIGEN (BB-1) EXPRESSED ON EPSTEIN-BARR VIRUS-ACTIVATED B CELL BLASTS, B LYMPHOBLASTOID CELL LINES, AND BURKITT'S LYMPHOMAS.

Yokochi T, Holly RD, Clark EA
(c/o Clark) Regional Primate Res. Center and Dept. Genetics, Univ. Washington, Seattle, WA, 98195
J Immunol; 128(2):823-827 1982

Two new cell surface antigens expressed on B lymphoblastoid cell lines (B-LCL) were defined with cytotoxic mouse monoclonal antibodies. One marker, BB-1 (for B lymphoblast antigen-1), was detected on human and nonhuman primate B-LCL, Epstein-Barr virus (EBV)-activated B cell blasts, most Burkitt's lymphomas, and Ia+ B lymphoblast-like myelomas. Polyclonal B cell activators such as pokeweed mitogen (PWM) and lipopolysaccharide (LPS) also induced the expression of BB-1 on immunoglobulin (Ig)-positive cells.In contrast, BB-1 could not be detected on normal lymphoid tissues by complement-dependent cytotoxicity and immunofluorescence (IF) assays or by analysis with a fluorescence-activated cell sorter (FACS). T cell blasts, T cell leukemias, and pre-B cell or erythroblastic leukemia cell lines were also BB-1 negative. Of particular interest was the finding that BB-1 was expressed on the Jijoye lymphoma but only marginally on a subline of Jijoye, P3HR-1, that lacks receptors for EBV and produces a defective virus incapable of transforming lymphocytes. A second lymphoblast antigen (LB-1) unlike BB-1, was present on both T and B cell blasts and virus-transformed T- and B-LCL but not on normal lymphoid tissues. (Author abstract) (50 Refs)

45. IDENTIFICATION OF EPSTEIN-BARR VIRUS STRAIN DIFFERENCES WITH MONOCLONAL ANTIBODY TO A MEMBRANE GLYCOPROTEIN.

Qualtiere LF, Chase R, Vroman B, Pearson GR
(c/o Pearson) Dept. Cell Biology and Microbiology, Mayo Foundation, Rochester, MN, 55901
Proc Natl Acad Sci USA; 79(2):616-620 1982

Two monoclonal antibodies directed against Epstein-Barr virus (EBV)-induced membrane antigens (MA) were isolated in this study. One of the monoclonal antibodies, designated 2F5.6, was an IgG2 which, as detected by membrane and fixed cell immunofluorescence, reacted with MA-positive lymphoblastoid cell lines that produced transforming EBV but not with the MA-positive P3HR-1 cell line that produced the lytic, non-transforming strain of this virus. This antibody precipitated the Mr 320,000/350,000 glycoprotein from B-95 virus infected cultures and the Mr 300,000 and 220,000/250,000 glycoproteins from Raji cells superinfected with P3HR-1 virus but did not precipitate any of these EBV-specific glycoproteins from the P3HR-1 cell line. In contrast, the second monoclonal antibody, IgM designated B10.3, reacted with all virus-producing cell lines including the P3HR-1 cell line. The identity of the glycoprotein that serves as the target for this antibody is still unknown. Neither antibody had neutralizing activity against the B-95 or P3HR-1 strain of EBV. These results indicated that the 2F5.6 monoclonal antibody was directed against an antigenic determinant on the major membrane glycoprotein which is common to transforming strains of EBV but absent from the lytic P3HR-1 strain whereas the B10.3 monoclonal antibody

was directed against a group-specific EBV-induced membrane determinant. (Author abstract) (25 Refs)

OTHER RELATED STUDIES

46. POLY(ADP-RIBOSE) HAS A BRANCHED STRUCTURE IN VIVO.

Juarez-Salinas H, LeviV, Jacobson EL, Jacobson MK
Dept. Chemistry, North Texas State Univ./Texas Coll. Osteopathic Medicine, Denton, TX, 76203
J Biol Chem; 257(2):607-609 1982

We have searched for the presence of branching in the chromosomal polymer poly(ADP-ribose) as it occurs in vivo. Treatment of the polymer with phosphodiesterase and phosphomonoesterase results in the conversion of internal residues to the nucleoside ribosyladenosine and the conversion of points of branching to diribosyladenosine. We have detected diribosyladenosine in digests of the polymer derived from carcinogen-treated SV40 virus-formed 3T3 cells and in normal rat liver, kidney, and spleen. The frequency of residues involved in branching varied from 0.8 to 1.6 mole % over a 50-fold range of total levels of poly(ADP-ribose). Thus, branching seems to be a general feature of poly(ADP-ribose) as it occurs in vivo. (Author abstract) (17 Refs)

47. ANTIVIRAL, ANTIMETABOLIC, AND CYTOTOXIC ACTIVITIES OF 5-SUBSTITUTED 2'-DEOXYCYTIDINES.

De Clercq E, Balzarini J, Descamps J, Huang GF, Torrence PF, Bergstrom DE, Jones AS, Serafinowski P, Verhelst G, Walker RT
Rega Inst. Medical Res., Katholieke Universiteit Leuven, B-3000 Leuven, Belgium
Mol Pharmacol; 21(1):217-223 1982

Several 5-substituted-2'-deoxycytidines (including 5-bromo-dCyd, 5-iodo-dCyd, 5-nitro-dCyd, 5-ethynyl-dCyd, 5-propyl-dCyd, (E)-5-(2-bromovinyl)-dCyd, (E)-5-(2-iodovinyl)-dCyd) were evaluated for their effects on herpes simplex virus (HSV: type 1 + type 2) and vaccinia virus cytopathogenicity and multiplication in primary rabbit kidney (PRK) cell cultures. The effects of 5-substituted 2'-deoxycytidines on incorporation of radiolabeled precursors into DNA of PRK or murine L1210 cells were studied and incorporation pathways hypothesized. L1210 tumor cell growth under the influence of the compounds was examined. The 5-X-deoxyuridine derivatives that are not selective as antiherpes agents became specific inhibitors of HSV replication when converted to dCyd counterparts, whereas 5-X-dUrd derivatives that are highly selective in antiherpes activity retained their specificity after amination to dCyd analogues. The compounds were relatively weak against tumor cell proliferation. (31 Refs)

48. IDENTIFICATION OF A BALB/C H-2LD GENE BY DNA-MEDIATED GENE TRANSFER.

Goodenow RS, McMillan M, Orn A, Nicolson M, Davidson N, Frelinger JA, Hood L
Div. Biology, California Inst. Technology, Pasadena, CA, 91125
Science; 215(4533):677-679 1982

Gene transfer and immunoselection were used in the identification of a BALB/c genomic clone containing an H-2Ld gene (clone 27.5). Transformation of thymidine kinase-negative C3H mouse L cells with the cloned 27.5 DNA together with the herpes simplex virus tk gene produced transformants expressing Ld molecules detected by radioimmune assay with monoclonal hybridoma antibodies to Ld antigens. The foreign Ld gene products expressed by cloned mouse L cell transformants were shown to be virtually indistinguishable from BALB/c spleen Ld molecules by two-dimensional electrophoretic analysis of H-2Ld immunoprecipitates. These results indicate that the genomic clone 27.5 contains a functional BALB/c H-2Ld gene and demonstrate the usefulness of this approach for identifying the gene products encoded by cloned genes which are members of a multigene family. Furthermore, the ability to place cell-surface recognition molecules on the surfaces of foreign cells provides a powerful opportunity for functional analyses of these molecules. (Author abstract) (20 Refs)

49. ADENO-ASSOCIATED VIRUS RNA TRANSCRIPTION IN VIVO.

Marcus CJ, Laughlin CA, Carter BJ
Lab. Experimental Pathology, Natl. Inst. Arthritis, Diabetes, Digestive and Kidney Diseases, NIH, 9000 Rockville Pike,, Bethesda, MD, 20205
Eur J Biochem; 121(1):147-154 1981

We have studied RNA transcripts of the defective parvovirus, adeno-associated virus (AAV) present in poly-(A)rich and poly-(A)free fractions of nuclear and cytoplasmic RNA prepared from cells infected together with a helper adenovirus. Cytoplasmic poly-(A)rich RNA contains three overlapping spliced AAV RNAs having sizes of 3.9 x 10(3), 3.3 x 10(3) and 2.3 x 10(3) base respectively. The nuclear precursors of the RNAs appear to be the coterminal unspliced poly(A)-rich RNAs containing 4.2 x 10(3), 3.6 x 10(3) and 2.6 x 10(3) bases respectively. These unspliced RNAs were also found in the cytoplasm. The nuclear poly(A)-free RNA contained a heterogenous population of AAV RNAs that were generally smaller than 2.3 x 10(3) bases. In addition, the Hirt pellet fraction of the nuclear RNA contained two discrete AAV poly(A)-free RNAs having sizes of 2.5 x 10(3) and 2.8 x 10(3) bases. The 4.2 x 10(3) and 3.6 x 10(3)-base unspliced RNAs are more abundant than the coterminal 3.9 x 10(3) and 3.3 x 10(3)-base spliced RNAs whereas the 2.3 x 10(3)-base spliced RNA is much more abundant than the 2.6 x 10(3)-base unspliced RNA. Thus, the cytoplasmic abundance of the AAV spliced RNAs appears to be controlled in part by the post-transcriptional events of splicing or message stability. We also analyzed the effects of AAV defective-interfering genomes upon AAV transcription. These studies showed that when synthesis of standard AAV genomes was inhibited more than 10-fold by defective-interfering genomes there was no significant effect on the types or amounts of AAV RNA transcripts which accumulated. These observations indicate that interference by defective-interfering genomes occurs mostly at the level of DNA replication rather than transcription. (Author abstract) (24 Refs)

50. MULTIPLICITY ACTIVATION OF HERPES SIMPLEX VIRUS IN MOUSE NEUROBLASTOMA (C1300) C ELLS.

Vahlne A, Nilheden E, Svennerholm B
Dept. Virology, Inst. Medical Microbiology, Univ. Goteborg, Guldhedsgatan 10B, S-413 46 Goteborg, Sweden
Arch Virol; 70(4):345-356 1981

The virus yields and number of infectious centers of HSV infected mouse neuroblastoma C1300 cells (clone 41 A3) infected at different multiplicities of infection (MOI) were found to vary more than the differences of HSV concentrations of the virus suspensions used for infection of the cells. This suggested that a C1300 cell had to be infected with more than one HSV particle in order to produce progeny virus-multiplicity activation. The greater than expected enhancement of virus produc-

tion of C1300 cell cultures receiving increasing MOI of HSV was probably not due to improved virus adsorption, nor influenced by non-virus factors in the virus inoculum stimulatory for HSV replication. A hypothesis, that the block in virus replication was promoted by an inhibitor of an HSV specified regulatory protein and could be overcome by the addition of HSV DNA copies in the infected cell, was supported by the results of two types of experiments. Presence of phosphonoformic acid, an inhibitor of the HSV specified DNA polymerase, in the culture medium of HSV infected permissive GMK cells resulted in non-linear relationships between virus yields and MOI. An HSV temperature sensitive mutant (ts B5), defective in a late structural protein, rescued wild type HSV in C1300 cells. (Author abstract) (24 Refs)

51. ADENOVIRUS ADSORPTION AND STEROL REDISTRIBUTION IN KB CELL PLASMA MEMBRANE.

Hennache B, Torpier G, Boulanger P
Laboratoire de Virologie Moleculaire (U.233), INSERM, 59045 Lille, France
Exp Cell Res; 137(2):459-463 1982

Filipin was used as a chemical probe for localization of sterols in freeze-fractured plasma membrane of KB cells. After adenovirus particle adsorption, marked changes occurred in the number and planar distribution of sterols and of intramembranous particles (IMPs). Filipin-sterol complexes became more abundant and both sterols and IMPs aggregated in a network pattern. It was suggested that redistribution of sterols and rearrangement of IMPs were interconnected phenomena, which represented an early cellular response to adenovirus attachment. (Author abstract) (23 Refs)

52. CHEMICAL CARCINOGENS TRANSFORM BHK CELLS BY INDUCING A RECESSIVE MUTATION.

Bouck N, Di Mayorca G
Dept. Microbiology/Immunology, Northwestern Univ., Chicago, IL, 60611
Mol Cell Biol; 2(2):97-105 1982

Mechanisms of transformation (TF) by chemical carcinogens were studied in the baby hamster kidney cell line BHK-21 clone 13. Tumorigenic transformed cell lines were derived from BHK cells by exposure to papovavirus (PV), dimethylnitrosamine (DMN), nitrosomethylurea (NMU), or 4-nitroquinoline-N- oxide (4-NQO) or by spontaneous transformation. NMU or 4-NQO produced TF and mutations to 6-thioguanine-resistance (6-TGR) or ouabain-resistance (OR) in a dose-related fashion. Parallel dose-response curves for TF, 6-TGR, and OR suggested that TF and mutation occurred by similar mechanisms. Two frame-shift mutagens, ICR-170 and ICR-191, had dose-response curves for survival and TF that were identical. Both compounds produced temperature-restriction mutations in inverse proportion to frame-shift mutations. Fusion of transformed cells with isogeneic normal cells showed that TF induced by PV behaved as a genetic dominant, but spontaneous or chemically induced TF behaved as a genetic recessive. These genetic patterns were exhibited soon after fusion and also after establishment of hybrid clones. All hybrids were nearly tetraploid. Hybrids showed segregation of TF; transformed phenotype was reexpressed in most cases in the form characteristic of the original transformed parent. Results indicate that chemical carcinogenesis in BHK cells involves induction of a recessive mutation as part of a multistage process. (58 Refs)

53. EFFECTS OF N-BUTYRATE ON EPSTEIN-BARR VIRUS-CARRYING LYMPHOMA LINES.

Anisimova E, Saemundsen AK, Roubal J, Vonka V, Klein G
(c/o Saemundsen) Dept. Tumor Biology, Karolinska Institutet, S104 01 Stockholm, Sweden
J Gen Virol; 58(part1):163-171 1982

The compound n-butyrate has been shown to induce Epstein-Barr virus (EBV) antigen synthesis in certain EBV-carrying lymphoma lines. We have studied the effect of n-butyrate on two EBV-positive Burkitt lymphoma lines by immunofluorescence and electron microscopy. In the producer line P3HR-1, the drug induced not only early antigen (EA) and virus capsid antigen (VCA) synthesis, as shown before, but also increased the number of cells containing virus particles. The transition from VCA expression to the formation of virus particles was much more effective in treated cells than in EBV antigen-producing cells of the same line. The productive cycle was associated with the development of characteristic morphological changes. In the non-producer Raji cells, n-butyrate induced EA in only a minor fraction of the cells. There were, however, clear signs of differentiation in the direction of plasma cells. Two days after the addition of n-butyrate 80% of the Raji cells could be classified as plasmablasts. After 72 hr, 20% of the cells appeared as typical plasma cells. (Author abstract) (33 Refs)

54. LOGISTICS OF TUMOR IMMUNOLOGY.

Kirchner H
Inst. Virus Res., German Cancer Res. Center, Im Neuenheimer Feld 280, D-6900 Heidelberg 1, W. Germany
Klin Wochenschr; 60(1):37-47 1982

This article deals with the experimental problems of tumor immunology. It stresses that animal tumors should in their pathogenesis closely reflect the pathogenesis of human cancer because o therwise they may be useless and cloud the issues instead of making useful contributions. The article lists all the problems of studying the immunobiology of human cancer which appear to be insurmountable at this time. The development of human cancer appears to be a multistep process which takes many years (decades) before the disease becomes manifest. The respective roles of chemical carcinogens, viruses or other damages are uncertain at present, but perhaps viruses do play a role as initiators in early life as suggested by the evidence obtained for the Epstein-Barr virus. If the hypothetical considerations of the role of a virus in tumor initiation and role of nonspecific defense mechanism in antitumor surveillance will receive further support, studies of primary antiviral defense mechanisms will turn out to be of utmost importance. In the conclusions, we outline that it is more important to reconcile the theories of 'tumor immunology' with the concepts of carcinogenesis than with the concepts of general immunology. The latter are, of course, of high biologic interest and of great importance but they may not apply to the pathogenesis of tumors, and immunologic mechanisms may not contribute to the defense against cancer. (Author abstract) (66 Refs)

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VIRUS ISOLATION/ STRUCTURE/ CHARACTERIZATION/ IDENTIFICATION

1. GROUPING OF ADENOVIRUSES AND IDENTIFICATION OF RESTRICTION ENDONUCLEASE CLEAVAGE PATTERNS OF ADENOVIRUS DNAS USING INFECTED CELL DNA: SIMPLE AND PRACTICAL METHODS.

Suzuki N, Ueno T, Yamashita T, Fujinaga K
Dept. Ophthalmology, Sapporo Medical Coll., S-1, W-16, Chuo-ku, Sapporo, Hokkaido 060, Japan
Microbiol Immunol; 25(12):1291-1301 1981

Simple and practical methods are described for determination of grouping of adenoviruses and identification of viral DNA cleavage patterns, using infected cell DNA. Grouping of adenovirus DNAs by means of infected cell DNA was performed with the spot hybridization technique. Results were similar whether viral or infected cell DNA was used. Restriction endonuclease cleavage patterns could be obtained by Southern blot hybridization, using infected cell DNA. The method was very sensitive and permitted identification of the cleavage pattern of the viral DNA of the inoculum by means

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of DNA extracted from infected cells at a stage when cytopathic effect was undetectable. Ethidium bromide staining of gels without Southern blot hybridization permits precise detection of the endonuclease cleavage pattern of viral DNA, in spite of background staining due to cellular DNA. However, the ethidium bromide staining procedure required about 100-fold more infected cells than the Southern blot procedure. The preparation of infected cell DNA used in these procedures was technically much easier than that of viral DNA. Only small numbers of infected cells are required so that many isolates may be investigated with ease. (18 Refs)

2. RAPID TYPING OF HERPES SIMPLEX VIRUS BASED ON IMMUNOLOGICAL SPECIFICITY OF VIRAL THYMIDINE KINASE AND TYPING ACCORDING TO SENSITIVITY TO IODODEOXYURIDINE.

Gronowitz JS, Kallander CF, Jeansson S, Wallin J
Dept. Medical Virology, Uppsala Univ., Biomedical Center, 751 23 Uppsala, Sweden
J Clin Microbiol; 15(3):366-371 1982

Iododeoxyuridine sensitivity test for discriminating between herpes virus simplex (HVS) Type 1 and 2 strains and typing procedure based on immunological specificity of the thymidine kinase (dTK) present in the first passage of the virus isolate is described. The latter method can be used for typing HVS dTK present in the vesicle fluid, allowing the verification of HVS infection and determination of HVS Type within 5 hr. The possibility of detecting therapy-resistant HVS mutants with this methods is illustrated. (13 Refs)

3. RAPID SEROLOGICAL TECHNIQUE FOR TYPING HERPES SIMPLEX VIRUSES.

Cleveland PH, Richman DD, Oxman MN, Worthen DM
Res. Service, Veterans Administration Medical Center, Univ. California, San Diego, CA, 92161
J Clin Microbiol; 15(3):402-407 1982

A rapid serological technique for typing herpes simplex viruses (HSV) is described. The sensitive 125I-labeled staphylococcal protein immunoassay in which viral antigens are nonspecifically immobilized on filter paper disks in an immunofiltration manifold was adapted to the typing of HSV isolates. The sensitivity of the technique permits the assay to be carried out with a small number of infected cells and to be performed directly with the primary isolate. (28 Refs)

4. DNA OF HERPESVIRUS PAN, A THIRD MEMBER OF THE EPSTEIN-BARR VIRUS-HERPESVIRUS PAPIO GROUP.

Heller M, Gerber P, Kieff E
Majorie B. Kovler Lab., Univ. Chicago, Chicago, IL, 60637
J Virol; 41(3):931-939 1982

The DNA of herpesvirus pan, a primate B-lymphotropic herpesvirus, shares about 40% well-conserved sequence relatedness with Epstein-Barr virus (EBV) and herpesvirus papio DNAs. Labeled cl oned fragments from the EBV recombinant DNA library were cross hybridized to blots of EcoRI, XbaI, and BamHI restriction endonuclease fragments of herpesvirus pan genome. Regions of colinear homology were demonstrated between 6 x 10(6) daltons and 108 x 10(6) daltons in the DNAs. The structural organization of herpesvirus pan DNA was similar to the format of Epstein-Barr virus and herpesvirus papio DNAs. The DNA consists of two domains of largely unique sequence complexity, a segment Us of 9 x 10(6) daltons and a segment UL of 88 x 10(6) daltons. Us and UL are separated by a variable number of tandem repetitions of a sequence IR (2 x 10(6) daltons). There was homology between DNA which mapped at 26 to 28 x 10(6) daltons and 93 to 95 x 10(6) daltons in UL. The terminal reiteration component, TR, of herpesvirus pan DNA and sequences which mapped to the left of 6 x 10(6) daltons and to the right of 108 x 10(6) daltons had no detectable homology with the corresponding regions of Epstein-Barr virus DNA. (Author abstract) (39 Refs)

5. PURIFICATION OF GLYCOPROTEIN GD OF HERPES SIMPLEX VIRUS TYPES 1 AND 2 BY USE OF MONOCLONAL ANTIBODY.

Eisenberg RJ, Ponce de Leon M, Pereira L, Long D, Cohen GH
Dept. Pathobiology, Sch. Veterinary Medicine, Univ. Pennsylvania, Philadelphia, PA, 19104
J Virol; 41(3):1099-1104 1982

Glycoproteins gD-1 and gD-2 of herpes simplex virus Types 1 and 2, respectively, were purified on an immunoadsorbent consisting of the type-common monoclonal antibody HD-1 linked to Sepharose. Each glycoprotein was of sufficient purity, quantity, and biological activity to be used for immunological and biochemical studies. Each glycoprotein induced high titers of type-common monospecific neutralizing antibody in mice. Amino acid analysis indicated that gD-1 and gD-2 had similar though not identical amino acid compositions. (Author abstract) (22 Refs)

6. SULPHATED GLYCOPROTEINS INDUCED BY HERPES SIMPLEX VIRUS.

Hope RG, Palfreyman J, Suh M, Marsden HS
Medical Res. Council Virology Unit., Inst. Virology, Univ. Glasgow, Church St., Glasgow G11 5JR, Scotland
J Gen Virol; 58(part2):399-415 1982

BHK cells infected with strain 17 syn+ (HSV-1) or HG52 (HSC-2) incorporated inorganic sulfate into polypeptides which co-migrated on SDS-polyacrylamide gels with virus-induced glycoproteins. The major sulfated glycoprotein was glycoprotein E. In addition, less-intense sulfated bands co-migrated with glycoprotein D and HSV-1 glycoprotein A/B/C. Sulfate label co-migrating with HSV-2 glycoprotein A/B/C was occasionally observed. We have investigated which sulfated polypeptides are excreted from infected cells. Major ones of apparent mol wt 32,000, 34,000 and 35,000 were excreted from cells infected with 17 syn+. In addition, polypeptides which migrated in the vicinity of glycoprotein D were oft en excreted from cells infected with either 17 syn+ or HG52. The 32K, 34K and 35K polypeptides were antigenically related to glycoprotein D and over 95% of the total amount synthesized was excreted. Analysis of intracellular sulfated polypeptides using intertypic recombinants mapped glycoprotein E to between 0.832 and 0.950 units of the HSV genome. (Author abstract) (39 Refs)

7. SOLID-PHASE IMMUNE ELECTRON MICROSCOPY-DOUBLE-ANTIBODY TECHNIQUE FOR RAPID DETECTION OF PAPOVAVIRUSES.

Giraldo G, Beth E, Lee J, De Harven E, Chernesky M
NCI, Naples, Italy
J Clin Microbiol; 15(3):517-521 1982

Detection of a papovavirus by a solid-phase immune electron microscopy-double-antibody technique is described. The technique takes advantage of a highly enhanced immunoadsorption of the virus to Formvar-carbon-coated grids pretreated with

the corresponding antiserum and of a further specific 'decoration' or coating of the virus particles by the same antiserum. The procedure was defined with BK virus and tested for specificity with mouse polyomavirus. (24 Refs)

8. VIRAL PARTICLES IN A PAPILLOMA FROM A GREEN LIZARD (LACERTA VIRIDIS).

Cooper JE, Gschmeissner S, Holt PE
Dept. Pathology, Royal Coll. Surgeons of England, 35-43 Lincoln's Inn Fields, London, WC2A 3PN, England
Lab Anim; 16(1):12-13 1982

Papillomata are well recognized in lizards, particularly those of the genus Lacerta. They appear to be transmissible by contact and are believed to be due to a virus. However, the only successful attempt to identify or isolate a viral agent was that by Raynaud and Adrian (1976), using electron microscopy, who described particles resembling herpesviruses, reoviruses and papovaviruses, in a green lizard (Lacerta viridis). In this paper we report the detection of viral particles in electron micrographs of a papilloma from the same species. (Author abstract) (5 Refs)

VIRUS ASSOCIATED CELL TRANSFORMATION AND HOST ALTERATION

9. THE HANDBOOK OF CANCER IMMUNOLOGY: VOLUME 8: TUMOR ANTIGENS: STRUCTURE AND FUNCTION.

Waters H
NIH, Bethesda, MD
The Handbook of Cancer Immunology: Volume 8: Tumor Antigens: Structure and Function. New York, Garland STPM Press, 418 pp., 1981.

Structural and functional aspects of tumor antigens are reviewed. Mechanisms of reactions between lymphocytes and antigens are discussed. Characteristics of Ia antigens in mice and humans are presented and the significance of these antigens is considered. Histocompatibility antigens and tumor-associated antigens in plasma and urine are discussed and their diagnostic significance evaluated. Molecular structure and mechanisms of antibody-antigen interactions are outlined. Other topics include the use of cell membranes to assess phenotypic expression, masking of cancer-cell surface antigens, cell surface modulation in mammary cancer, antigen activation of T cells, assay and structure of carcinoembryonic antigens, induction of immune response to tumors induced by simian virus 40, and tumor-associated immunosuppressive serum proteins. Concentration and distribution of antigens are related to normal and pathological conditions. Discussion includes review of literature, presentation of original findings, and speculation on future research efforts. Converging and diverging viewpoints are expressed. (1659 Refs)

10. GENETICAL MECHANISMS OF CARCINOGENESIS. THE 'ONCOGENETICS' PROGRAM.

Shapiro NI
No affiliation given
Vestn Akad Nauk SSSR; (12):69-75 1981

Current research in the field of somatic cell genetics is reviewed. Applications of the somatic cell genetics methods to the study of the mechanisms of virus carcinogenesis showed that simian virus 40 (SV40) and adenovirus Type 3 induced gene mutation in chinese hamster cells. The mutagenic effect of DNA-containing oncogenic viruses was not locus-specific. Both mutagenesis and neoplastic transformation of cells were mediated via the same mechanism of incorporation of SV40 genome into genome of mammalian cells. (no Refs) (In RUS)

11. VIRAL GENE PRODUCTS IN ADENOVIRUS TYPE 2-TRANSFORMED HAMSTER CELLS.

Esche H
Inst. Genetics, Univ. Cologne, Cologne, W. Germany
J Virol; 41(3):1076-1082 1982

I have analyzed viral gene products expressed in five adenovirus Type 2 (Ad2)-transformed hamster embryo cell lines (Ad2 HE1-5) by cell-free translation of cytoplasmic, viral RNA which was selected by hybridization to cloned restriction endonuclease fragments of Ad2 DNA. Proteins synthesized in vitro were analyzed by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels and compared with those directed by RNAs prepared from productively infected cells. The early regions E1 and E4 of adenovirus Type 2 (Ad2) were found to be expressed in all of five Ad2-transformed hamster embryo cell lines. RNA transcribed from early region E2, which codes for the 72,000-mol-wt (72K) DNA-binding protein was detected in cell line HE1 only, and early region E3 was expressed exclusively in cell line HE4. RNA transcribed from the region between approx 12 and 35 map units, coding for immediate early (13.5K, 52/53K) and intermediate early proteins (13.6K, 16K, 17K, 87K), as well as RNA from late genes, was not found in any of the cell lines. The proteins synthesized in vitro by RNA prepared from cell lines HE1 to HE5 had electrophoretic mobilities similar to those programmed by RNA from productively infected cells. (Author abstract) (23 Refs)

12. MULTIPLE TRANSFORMATION PHENOTYPES AMONG REVERTANTS OF TEMPERATURE-SENSITIVE MUTANTS IN THE TYPE 5 ADENOVIRUS DNA-BINDING PROTEIN.

Carter TH, Nicolas JC, Young CS, Fisher PB
Dept. Biological Sciences, St. John's Univ., Jamaica, NY, 11439
Virology; 117(2):519-521 1982

Seven independently isolated revertants of temperature-sensitive mutants in the 72K protein of Ad5 were tested for the ability to transform rat cells under a variety of conditions. Using the continuous cell line designated CREF, at 36 C the range of transformation phenotypes of the different revertants included a frequency characteristic of WT and also the elevated frequency associated with the parental temperature-sensitive alleles. Transformation frequency did not correlate with other phenotypes, such as plaque size or plaquing efficiency on HeLa cells at 38.5-39 C. Therefore, although it is likely that the 72K protein modulates transformation, it is possible to dissociate genetically this regulatory function of the protein from its replicative function in permissive infection. (Author abstract) (11 Refs)

13. ADENOVIRUS E1B-58KD TUMOR ANTIGEN AND SV40 LARGE TUMOR ANTIGEN ARE PHYSICALLY ASSOCIATED WITH THE SAME 54 KD CELLULAR PROTEIN IN TRANSFORMED CELLS.

Sarnow P, Ho YS, Williams J, Levine AJ
State Univ. New York, Stony Brook, NY, 11794
Cell; 28(2):387-394 1982

The adenovirus E1b-58kd tumor antigen has been detected in a physical association with a 54 kilodalton cellular protein in adenovirus-transformed mouse cells. Antibody specific for the E1b-58kd protein coimmunoprecipitates a 54 kd protein from transformed, but not from productively infected, cells. Monoclonal antibody specific for the cellular 54 kd protein coimmunoprecipitates the adenovirus E1b-58kd protein from transformed cell extracts. The same or closely related cellular 54 kd protein, associated with the adenovirus E1b-58kd protein, was present in the SV40 large T antigen-54 kd complex previously detected in SV40-transformed mouse cells. The identity of the 54 kd protein is based on the immunological specificities of the anti-54 kd monoclonal antibodies and partial peptide maps of the 54 kd protein associated with the adenovirus and SV40 tumor antigens. The adenovirus E1b-58kd-54 kd complex, like the SV40 large T antigen-54 kd complex, is heterogeneous in size or mass. While all of the cellular 54 kd protein in the adenovirus-transformed cell extract is found in a complex with the E1b-58kd protein, some of the viral 58 kd antigen is detected in a form not associated with the 54 kd protein. The fact that the adenovirus and SV40 tumor antigens, both required for transformation, can be found in physical association with the same cellular protein in a transformed cell is a good indication that these two diverse viral proteins share some common mechanisms or functions. (Author abstract) (43 Refs)

14. SPECTRUM OF TUMORIGENIC PHENOTYPES AMONG ADENOVIRUS 2-, ADENOVIRUS 12-, AND SIMIAN VIRUS 40-TRANSFORMED SYRIAN HAMSTER CELLS DEFINED BY HOST CELLULAR IMMUNE-TUMOR CELL INTERACTIONS.

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Natl. Inst. Allergy and Infectious Diseases, NIH, Bethesda, MD, 20205
Cancer Res; 42(3):939-944 1982

The tumor-inducing capacities of adenovirus Type 2-, adenovirus Type 12-, and SV40-transformed LSH hamster embryo cells define a spectrum of four distinct tumorigenic phenotypes: Type I, nononcogenic for newborn hamsters but oncogenic for nude mice; Type II, oncogenic for new born hamsters but nononcogenic in syngeneic adults; Type III, oncogenic for both newborns and syngeneic adults; and Type IV, almost equally oncogenic for syngeneic and allogeneic adult hamsters. Modulation of the cellular immune response of the recipient hamster by immunosuppression or by alloimmunization alters tumor susceptibility, suggesting that dynamic transformed cell-host cellular immune interactions determine the tumorigenic phenotype of a transformed cell line. There is no correlation between the immunogenicities of the transformed cell lines tested and their tumor-inducing capacities. However, a strong correlation exists between the ability of transformed hamster embryo cells expressing Phenotype IV to produce tumors in allogeneic CB hamsters and their resistance to cytolysis in vitro by activated macrophages. These data suggest that, in addition to transformation, DNA viruses may convey specific tumorigenic phenotypes to the cells that they infect by inducing cellular traits that determine the inherent susceptibility or resistance of a cell to host cell-mediated immune destruction. (Author abstract) (29 Refs)

15. PRODUCT OF ADENOVIRUS TYPE 2 EARLY GENE BLOCK E1 IN TRANSFORMED CELLS ELICITS CYTOLYTIC RESPONSE IN SYNGENEIC RATS.

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Virology; 117(2):530-535 1982

The cytotoxic immune response to three well-characterized adenovirus Type 2 (Ad2)-transformed cell lines (A2T8, 50A, and T2C4) was studied in syngeneic LIS rats. Antibodies against the three virus-transformed lines were raised by hyperimmunization. Cytolytic T cells were generated by in vivo immunization and in vitro education with A2T8 and 50A cells. The complement-dependent antibody-mediated cytotoxicity with hyperimmune sera, T-cell cytotoxicity, and antibody-blocking effects in T-cell cytotoxicity assays indicate that these three Ad2-transformed cell lines share specific antigen This observation was further confirmed by indirect immunofluorescence staining of the cell surface. Because it is absent in syngeneic LIS embryo fibroblasts and in syngeneic LIS cells transformed with EcoRI-C fragment of Ad12 DNA, this antigen appears to be Ad2 specific. Since the only Ad2 function shared and expressed by all three of these cell lines is the early gene block E1, it is concluded that its product or products elicit both humoral and T -cell cytolytic responses. (Author abstract) (17 Refs)

16. SHIFTS IN THE EXTENT AND PATTERNS OF DNA METHYLATION UPON EXPLANATION AND SUBCULTIVATION OF ADENOVIRUS TYPE 12-INDUCED HAMSTER TUMOR CELLS.

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Virology; 118(1):169-180 1982

Undifferentiated sarcomas were induced in hamsters by the injection of adenovirus type 12 (Ad12) into newborn animals. Among the survivors, tumor incidence was 71%: 66% in male animals, 75% in female animals. The tumors infiltrated local muscular tissue, and clusters of tumor cells could also be detected inside lymphatic vessels. DNA was extracted from 11 different tumors. The DNA preparations were analyzed for the patterns of integration of Ad12 DNA by restriction enzyme cleavage, Southern blotting, and DNA-DNA hybridization. These data provided direct evidence for the notion that the extent of DNA methylation was dependent on culture conditions. From one tumor line (H313) several independent subclones were established and the patterns of methylation in the tumor and in the subclones were compared. In clones maintained in conditioned medium, the patterns of methylation remained constant as compared to the original tumor cells. The low levels of viral DNA methylation in the tumors were not accompanied by an abundance of Ad12-specific RNA in the tumors. Thus, the undermethylation of integrated adenovirus genes in tumor and transformed cells appeared to be a necessary but not a sufficient precondition for the expression of these genes. (Author abstract) (27 Refs)

17. SMALL T PROTEIN OF SIMIAN VIRUS 40 IS REQUIRED FOR DENSE FOCUS FORMATION IN A RAT CELL LINE.

Sugano S, Yamaguchi N, Shimojo H
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J Virol; 41(3):1073-1075 1982

Incomplete foci were formed in a Fischer rat cell line infected with simian virus 40 deletion mutants encoding defective small t antigen. The foci were small and rarely piled up. The fraction of DNA-synthesizing cells in the foci was about half of that induced by wild-type virus. (Author abstract) (9 Refs)

18. DETECTION OF SIMIAN VIRUS 40 SURFACE-ASSOCIATED LARGE TUMOR ANTIGEN BY ENZYME-CATALYZED RADIOIODINATION.

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Int J Cancer; 29(3):337-344 1982

To facilitate detection of SV40 surface-associated tumor antigen (T-ag), conditions were established to surface label T-ag on intact cells by lactoperoxidase-catalyzed radioiodination (125I/LPO). SDS-PAGE analysis of anti-T immunoprecipitates of SV40-transformed and -infected cells labeled with 125I/LPO revealed the presence of iodinated T-ag. Several types of control experiments were employed t o guarantee the surface specificity of the 125I/LPO labeling technique. When SV40-transformed mouse cells were surface labeled with lactoperoxidase and glucose oxidase immobilized on insoluble beads, a preparation less readily internalized than soluble enzymes, T-ag was iodinated. Selective immunoprecipitation of surface antigens demonstrated that lactoperoxidase did not iodinate internally localized T-ag. A reconstruction experiment in which an extract of SV40-infected cells was added to uninfected cells prior to surface labeling suggested that T-ag released from lysed cells did not adhere significantly to monolayer surfaces and become iodinated. Finally, systematic omission of reactants from the iodination reaction revealed that exogenous addition of lactoperoxidase and H2O2 was necessary to generate an iodinated T-ag, indicating that endogenous host cell reactants do not contribute significantly to the iodination of T-ag. 125I-labeled T-ag was detectable on the surface of SV40 tsA-infected cells as the nonpermissive temperature 24 hr postinfection, indicating that the tsA lesion does not prevent the interaction of T-ag with the cell surface. When 125I/LPO-labeled transformed or infected cells were chased for 2.5 hr after labelling, iodinated T-ag was no longer associated with the cell monolayer but was immunoprecipitable from culture supernatants. Cultures from which labeled T-ag had been shed could then be relabeled with 125I/LPO and surface-associated T-ag was again detectable. These data suggest that surface-associated T-ag is continuously shed from the cell surface and is rapidly replaced in the membrane by intracellular T-ag. (Author abstract) (62 Refs)

19. ALTERATIONS IN MICROFILAMENTOUS AND MICROTUBULAR STRUCTURES ACCOMPANIED BY CELL GROWTH OF SV40-TRANSFORMED CLONAL MACROPHAGES, LINE 28-12 AND ITS SUBLINE.

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Cell Struct Funct; 6(4):329-339 1981

Morphological patterns of sets of actively growing and stationary simian virus 40 (SV40)-transformed macrophages of the clonal line 28-12, obtained from SV40-infected cultures of mouse bone marrow cells, were compared with those of the slowly growing subline 28-12(Ara) isolated from cytosine arabinoside-treated cultures. Transmission and scanning electron microscopic observations revealed microfilamentous and microtubular structures in the cells. Bundles of 70 A microfilaments were found in fully-transformed, actively growing 28-12 cells but not in minimally-transformed, stationary 28-12 cells, an observation which inconsistent with other studies. A decrease in microtubules apparently occurs in actively growing cells which have high levels of SV40 T-antigen, although evidence is not unequivocal. Response of the cells to colchicine shows that cytoskeletal structures, including microtubules, are affected by transformation. The 28-12(Ara) cells, which are considered moderately transformed cells, have a significant amount of SV40 T antigen, fewer microtubules than either type of 28-12 cells, lower response to colchicine, and slower growth, but the cells also have a phagocytic capacity. (32 Refs)

20. 12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA) INDUCES SISTER-CHROMATID EXCHANGES AND DELAYS IN CELL PROGRESSION IN CHINESE HAMSTER OVARY AND HUMAN CELL LINES.

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Mutat Res; 92(1/2):393-409 1982

In Chinese hamster ovary (CHO) cells and SV40-transformed human fibroblast cell lines (GM637 and XP12RO), 12-O-tetradecanoylphorbol-13-acetate (TPA: 1.0 nanog-1.0 ug/ml) caused a 20-45% increase in the frequency of sister chromatid exchanges (SCEs). This increase in SCEs was not caused by an impurity in the TPA preparation, or increased 5-bromo-2'-deoxyuridine uptake into DNA; it was independent of the incubation time between TPA exposure and fixation of the cells. Simultaneous exposure to TPA (1.0 ug/ml) and benzo(a)pyrene (BP: 1.7 x 10(-5)-3.3 x 10(-6) M) led to a synergistic SCE induction in CHO cells. No synergism was observed after simultaneous exposure to TPA (1 ug/ml) and mitomycin C (0.03 ug/ml) or UV light. In addition to its weak SCE-inducing effects in CHO cells, TPA alone caused a slight delay in cell cycle progression and markedly enhanced the cell cycle delay induced by BP. The SCE-inducing capacity of TPA in these cells was unrelated to its toxicity, because TPA was not toxic at the doses used. It also seemed to be unrelated to excision repair capacity, because TPA caused similar increases in SCE frequency in cells which were proficient (GM637), mildly deficient (CHO), and severely deficient (XP12RO) in excision repair. The significance of SCE induction and its relationship to other biologic effects of TPA remain unknown. (40 Refs)

21. DNA SEQUENCE HOMOLOGY AND CHROMOSOMAL DELETION AT A SITE OF SV40 DNA INTEGRATION.

Stringer JR
Cold Spring Harbor Lab., Cold Spring Harbor, NY, 11724
Nature; 296(5855):363-366 1982

The possibility that integration of simian virus 40 (SV40) might involve matching of short stretches of homologous sequences present in the otherwise heterologous SV40 and cellular genomes was explored in Fisher rat embryo fibroblasts that were transformed by SV40 infection (SVRE9 cells). A rat DNA fragment (9L) was sequenced and was shown to contain an unoccupied site of SV40 DNA integration. The SVRE9

cells were haploid for their single SV40 insertion. The putative unoccupied integration site was isolated from a library of endo:R:SstI-digested SVRE9 DNA. Analysis of Sst9.1 DNA by restriction endonuclea se digestion and blot hybridization localized the SV40 integration site to a 200-base pair endo:R:Sau3a fragment. SV40 DNA and rat DNA shared a 5-base pair sequence homology at a point coincident with the rat-SV40 junction in 9L DNA. The DNA sequence of the unoccupied SV40 integration site was repetitive, containing 12 thymine-guanine (T-G) pairs in tandem and composed almost entirely of T and G. A rearrangement of the rat genome, probably by a deletion of at least 3 kilobases, occurred at the site of SV40 integration. Thus, DNA sequence homology may mediate integrative recombination between SV40 DNA and the cellular chromosome. (25 Refs)

22. STABILITY OF POLYOMA DNA SEQUENCES AND VIRUS-CODED PROTEINS DURING TUMOR FORMATION.

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J Virol; 41(3):1000-1006 1982

To determine the stability of polyoma viral DNA in transformed rat cells during their growth in vivo, we compared the state and arrangement of polyoma virus DNA sequences in virus-transformed rat cell lines before and after their passage in vivo. In cell lines from 12 independent tumors induced by the inoculation of animals with three different transformed cell lines, we could detect no significant changes in the arrangement of viral DNA sequences associated with the in vivo passage of these cell lines. In 13 of 14 tumor cell lines examined, the pattern of polyoma virus tumor antigens, characterized by the presence of the polyoma virus large, middle, and small tumor antigens, was unchanged. (Author abstract) (23 Refs)

23. MONOCLONAL ANTIBODIES AGAINST POLYOMA VIRUS TUMOR ANTIGENS.

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Proc Natl Acad Sci USA; 79(4,part1):1059-1063 1982

A method is described for the production of hybridoma cell lines secreting antibodies directed against the polyoma virus tumor antigens (T-Ags). Ten cloned lines have been established by this procedure and designated alphaPy Cl and C4, with activities against all three T-Ags; alphaPy LT1,-4, and-7, with activities against large T-Ag only; and alphaPy MT5,-7,-10,-13, and-16, with activities against middle T-Ag only. Some of the antibodies appear to react also with specific cellular proteins. Data from studies on alphaPy MT7 point to the possible existence of more than one antigenic form of the viral middle T-Ag and, similarly, from studies on alphaPy LT7, to more than one form of large T-Ag. The approx positions on the polyoma virus genome of the sequences encoding antigenic determinants have been mapped by using defined polyoma virus deletion mutants. The availability of these monoclonal antibodies allows the isolation of large amounts of viral T-Ags. (Author abstract) (30 Refs)

24. POLYOMA MUTANTS THAT PRODUCTIVELY INFECT F9 EMBRYONAL CARCINOMA CELLS DO NOT RESCUE WILD-TYPE POLYOMA IN F9 CELLS.

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Proc Natl Acad Sci USA; 79(5):1479-1483 1982

Mouse embryonal carcinoma cells are refractory to infection by wild-type polyoma virus, the infection process apparently being blocked at a stage after adsorption and penetration but before early protein synthesis. Polyoma virus mutants capable of productive infection of mouse embryonal carcinoma cells have been isolated and these mutants all have DNA sequence alterations in a noncoding region near the origin of replication of the viral genome. PyF101 and PyF441 are two mutants selected for their ability to infect the embryonal carcinoma cell line F9. Here we show that these PyF mutants do not rescue replication of wild-type polyoma during a mixed infection of F9 cells. The mutant and wild-type DNAs were distinguished on the basis of restriction fragments obtained by digestion with Msp I or BstNI, and no wild-type DNA was detected in F9 cells coinfected with wild-type polyoma and with either PyF101 or PyF441. The mutant viruses do not appear to inhibit wild-type replication during a mixed infection because both mutant and wild-type DNAs can replicate efficiently in coinfected 3T6 cells which are permissive for both mutant and wild-type viruses. A double mutant having the PyF101 mutation and the ts-25E temperature-sensitive mutation in polyoma large tumor antigen was constructed and found to be temperature-sensitive for replication in F9 cells. This double mutant, designated PyFts-1, can be rescued in F9 cells at the restrictive temperature by coinfection with PyF441. These results suggest that the PyF mutations affect two processes in F9 cells, one involving expression of polyoma early genes and a second involving viral DNA replication. (Author abstract) (34 Refs)

25. INDUCTION OF SISTER CHROMATID EXCHANGE BY POLYOMA LARGE VIRAL TUMOR ANTIGEN IN TRANSFORMED RAT FIBROBLASTS.

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Cancer Res; 42(5):1909-1912 1982

The frequency of sister chromatid exchange (SCE) was determined in rat fibroblasts transformed by wild-type polyoma virus or by a mutant temperature sensitive for viral large tumor antigen function (ts-a). Elevated SCE frequencies were observed in two wild-type transformed cell lines growing at 37 C and in four ts-a-transformed lines upon growth at the permissive temperature for large viral tumor antigen (33 C). The increase in SCE frequency in ts-a-transformed cells at 33 C was reversed by growth at 39 C (nonpermissive for T-antigen function). An increase in SCE at 33 C was not observed in untransforme d cells or in a ts-a-transformed cell line which makes a defective large viral tumor antigen. These results suggest that large viral tumor antigen can induce SCEs. Since large viral tumor antigen is also responsible for amplification of integrated viral DNA sequences (4), we tried to correlate this phenomenon with the increased SCE frequency. However, increasing SCE artificially by growing cells in the presence of 12-O-tetradecanoylphorbol-13-acetate did not result in amplification of integrated viral DNA in the absence of large viral tumor antigen function. Thus, there is no simple causal relationship between increased SCE and amplification. (Author abstract) (18 Refs)

26. TRANSFORMATION-DEPENDENT LOSS OF THE HYALURONATE-CONTAINING COATS OF CULTURED CELLS.

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J Cell Physiol; 110(2):123-128 1982

Coats of a variety of cell types, including a rat fibrosarcoma (RFS) cell line which is known to synthesize large amounts of hyaluronate, and two sets of parent and virus-transformed cell lines [Swiss 3T3 vs Simian virus-3T3 cells and baby hamster kidney cells (BHK) vs polyoma-BHK] were studied. Cell coat size was determined by comparing the pattern of settled RBC on control cultures with that of streptomyces hyaluronidase-treated cultures. The cell coat of RFS cells was quite large, as much as 25 um thick in places. The coats of 3T3 and BHK cells were also readily apparent although somewhat smaller than that of RFS cells. In virus-transformed cells, the coats were either greatly reduced or absent. RFS cells had the largest amount of hyaluronate followed in order by the 3T3 and the BHK cells. The two virus-transformed cell lines had much less hyaluronate than their parental counterparts. Differences between the amounts of hyaluronate were also reflected in the amounts of hyaluronate secreted into the medium. Cell lines with large coats tended to retain a greater fraction of total hyaluronate at their surfaces. In chemical studies of cells grown in the presence of 3H-glucosamime, the specific activity of the hyaluronate varied considerably from cell line to cell line, with the highest values being obtained from virus-transformed cells. The results indicated that, at least in the case of 3H-glucosamine, incorporation of radioactivity is not a reliable index of the net production of hyaluronate. It was concluded that virus transformation leads to a decrease in the size of the hyaluronate coat of 3T3 and BHK cells. (33 Refs)

27. CHARACTERIZATION OF A POLYPEPTIDE PRESENT IN HERPES SIMPLEX VIRUS TYPE 2-TRANSFORMED AND -INFECTED HAMSTER EMBRYO CELLS.

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J Virol; 41(3):1095-1098 1982

Transformation of hamster embryo cells by herpes simplex virus stimulated the production of a 35-kilodalton (35K) protein that was specifically immunoprecipitated, along with other polypeptides, by rabbit hyperimmune serum. This 35K polypeptide was further analyzed by partial digestion with Staphylococcus aureus V8 protease in parallel with a 35K polypeptide from herpes simplex virus Type 2-infected cells. These polypeptides had almost identical partial-proteolytic cleavage maps, indicating that they are probably the same or that they are very similar polypeptides. (Author abstract) (25 Refs)

28. INCIDENCE OF HERPES VIRUS (HSV-2) ALTERATIONS IN SQUAMOUS CELLS OF VAGINA EPITHELIUM.

Djelmis J, Zrnic S
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Zentralbl Gynaekol; 103(21):1309-1314 1981

The authors have analyzed 17,519 cytological cervix smears. Alterations characteristic of HSV infection were recorded from squamous cells of vaginal and cervical epithelia in 21 cases (0.12%). Incidences of cytologically detected HSV infection of the vagina and cervix were 0.17% among female factory workers and 0.08% among peasant women. No statistically unambiguous difference was found to exist between the incidences of genital HSV infections in the two groups examined (p greater than 0.05). The majority of women with genital HSV infections was between 21 and 25 yr old. (Author abstract) (17 Refs) (In GER)

29. EXPRESSION OF HERPES SIMPLEX VIRUS TYPE-COMMON SURFACE ANTIGENS IN CLONAL CELLS OF AN HSV TYPE 2-TRANSFORMED LINE: EFFECT OF ADRIAMYCIN.

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Dept. Periodontology and Endodontology, Sch. Dentistry, Tokushima Univ., Tokushima 770, Japan
Microbiol Immunol; 25(12):1265-1278 1981

The expression of herpes simplex virus (HSV) type-common surface antigen (CSA) was studied in a representative cell clone (155-4-03) of hamster cell line 155-4 transformed by HSV type 2. HSV-CSA was detected by the indirect immunofluorescence test. Expression of HSV-CSA was enhanced by treatment with inhibitors of RNA synthesis (adriamycin [ADM] and daunomycin) but not by inhibitors of DNA synthesis (2-iododeoxyuridine, bleomycin, mitomycin C and cytosine arabinoside), even though all of these agents decreased the number of viable cells in culture to about the same extent. Addition of puromycin or 2-deoxy-D-glucose to ADM markedly suppressed expression of CSA, indicating that both protein synthesis and glycosylation are necessary. ADM enhanced expression of CSA was sensitive to protease inhibitors (antipain and p-nitrophenyl-p'-guanidinobenzoate) and procaine. Procaine affects the organization of the cytoskeletal system (microfilaments and microtubules) in animal cells and also inhibits trypsin. Low concentrations of ADM (0.1 ug/ml) and actinomycin D (0.5 ug/ml) increased CSA expression in an additive manner, while optimal concentrations of each (0.25 and 2.0 ug/ml, respectively) did not. These findings indicate that the two drugs enhance CSA expression in the clone by a common mechanism. (40 Refs)

30. ALPHA2HS GLYCOPROTEIN BINDS TO LYMPHOCYTES TRANSFORMED BY EPSTEIN-BARR VIRUS.

Lewis JG, Crosier PS, Andre CM
Dept. Clinical Biochemistry, Christchurch Clinical Sch. Medicine, Christchurch Hosp., Christchurch, New Zealand
FEBS Lett; 138(1):37-39 1982

Binding of alpha2HS glycoprotein to Epstein-Barr virus (EBV)-transformed peripheral blood lymphocytes was studied by indirect immunofluorescence and by direct counts of cell-bound 125I-alpha2HS glycoprotein. This glycoprotein binds appreciably to EBV-transformed, but not to normal autologous, untransformed, lymphocytes (dissociation constant 5.0 x 10(-7) M, with 0.5 x 10(6) sites/cell). Binding of transformed cells is not blocked by deoxyribonuclease pretreatment, but can be partially blocked by ribonuclease or pronase pretreatment. (11 Refs)

31. SELECTION OF MOUSE CELLS PRODUCING HEPATITIS B SURFACE ANTIGEN AFTER TRANSFORMATION WITH CLONED VIRAL DNA.

Pourcel C, Dubois MF, Rousset S, Chany C, Tiollais P
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Prog Med Virol; 27:93-98 1981

Using cotransformation of Ltk- mouse fibroblasts with two plasmids bearing, respectively, two cloned Eco hepatitis B virus (HBV) DNA in tandem head to tail and cloned herpes virus (HSV) tk gene, mouse cell lines were selected which could synthesize hepatitis B surface antigen (HBsAg). All the Ltk+ selected clones synthesized HBsAg, indicating that they had received two plasmids. HBV DNA was integrated into high mol wt cellular DNA, and the original tandem structure was conserved. HBsAg synthesis in mouse cells showed that human viral gene transcription and translation was possible in the cells. (13 Refs)

VIRUS REPLICATION AND EXPRESSION

32. EXPRESSION OF A CLONED ADENOVIRUS GENE IS INHIBITED BY IN VITRO METHYLATION.

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Proc Natl Acad Sci USA; 79(4,part1):1073-1077 1982

In many viral and nonviral eukaryotic systems, an inverse correlation has been observed between the extent of DNA methylation at 5'-C-C-G-G-3' sites and the extent of expression of specific genes as mRNA. The E2a region of adenovirus serotype 2 (Ad2) DNA encodes the Ad2-specific DNA-binding protein required for viral DNA replication. In three lines of Ad2-transformed hamster cells (HE1, HE2, and HE3), multiple copies of the major part of the Ad2 genome persist in an integrated state. Cell lines HE2 and HE3 do not express the DNA-binding protein whereas line HE1 does so. It has been shown that, in cell line HE1, all 5'-C-C-G-G-3' (Hpa II/Msp I) sites in the E2a region remain unmethylated. Conversely, in lines HE2 and HE3 lacking expression of the E2a region all Hpa II sites are methylated. The cloned E2a region of Ad2 DNA, the HindIII A fragment in pBR322, was methylated in vitro by using Hpa II DNA methyltransferase (5'-C-C*G-G-3') or was left unmethylated. In vitro methylation did not break or nick supercoiled circular DNA. Methylated or unmethylated DNA was then microinjected into the nuclei of Xenopus laevis oocytes, and the subsequent synthesis of Ad2-specific RNA was monitored. In vitro-methylated DNA remained in the methylated state for 24 hr on microinjection into nuclei of Xenopus oocytes; unmethylated DNA remained unmethylated. When the injected DNA had been methylated by using Hpa II DNA methyltransferase, Ad2-specific RNA was not synthesized as late as 24 hr after microinjection. Unmethylated DNA was readily expressed into Ad2-specific RNA. As an internal control, unmethylated histone genes (h22 DNA) from sea urchin were microinjected together with methylated E2a DNA from Ad2. Ad2-specific RNA was not found; h22 DNA-specific RNA was readily detected. This finding ruled out nonspecific inhibitory effects in the methylated DNA preparation. It was also shown that transcription of the unmethylated HindIII A fragment of Ad2 DNA in Xenopus oocytes was initiated on the late promoter of the E2a region. The same promoter was used in productively infected KB cells. Methylation by BsuRI methylase (5'-G-G-C*C-3') did not inactivate the HindIII A fragment. These results provide evidence for the notion that methylated sequences at highly specific sites are involved in the regulation of gene expression. The actual nature of the regulatory signal is not yet understood. (Author abstract) (50 Refs)

33. DIFFERENT MRNAS FROM THE TRANSFORMING REGION OF HIGHLY ONCOGENIC AND NON-ONCOGENIC HUMAN ADENOVIRUSES.

Virtanen A, Pettersson U, Le Moullec JM, Tiollais P, Perricaudet M
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Nature; 295(5851):705-707 1982

Messenger RNA (mRNA) produced from the transforming region of DNA from human adenoviruses was characterized. Adenoviruses were cultured by infection of A549 cells derived from human oat-cell carcinoma. RNA was labeled with 32P. Complementary DNA (cDNA) was prepared from viral mRNA and hybridized to restriction enzyme fragments of viral DNA. The transforming region of viral DNA was characterized by inserting cDNA into pBR322 plasmids and hybridizing to labeled DNA fragments. Characterization of 38 clones containing sequences from the E1B region of the highly oncogenic Ad12 adenovirus showed three classes of mRNA. Ad12 mRNA were compared with mRNA from weakly (Ad5 and Ad7) and non-oncogenic (Ad2) adenoviruses. Results identified two donor sites, designated D1 and D2, and three acceptor sites, designated A1, A2, and A3, in the three classes of mRNA. Donor site D1 was well-conserved and generated a large splice in Ad12 and Ad5. Site D2 was well-conserved and generated a small splice in Ad5, Ad7, and Ad12. Site A1 generated a middle splice in Ad12 not found in Ad5. Site A2 was found in all three viral serotypes. Site A3 was found only in Ad7 and Ad12. Results indicate that mRNA from Ad12 have different structures from mRNA from Ad2, but suggest that the adenoviruses have evolved together. (30 Refs)

34. A BIOCHEMICAL INVESTIGATION OF THE ADENOVIRUS-INDUCED G1 TO S PHASE PROGRESSION: THYMIDINE KINASE, ORNITHINE DECARBOXYLASE, AND INHIBITORS OF POLYAMINE BIOSYNTHESIS.

Cheetham BF, Bellett AJ
Dept. Microbiology, John Curtin Sch. Medical Res., Australian Natl. Univ., Canberra, A. C. T., Australia
J Cell Physiol; 110(2):114-122 1982

Induction of thymidine kinase (TK) and ornithine decarboxylase (OD) was studied to explore the biochemistry of the G1 phase to S phase transition in Adenovirus type 5 (Ad5)-infected rat and C57 Black mouse embryo cells compared with the normal cell cycle. The inhibitors, actinomycin D (AD), alpha-methylornithine (AM), and methylglyoxal bis(guanylhydrazone) (MGBG), were used to show differences between the induction of TK by Ad5 and serum. In addition, three temperature-sensitive mutants of Ad5 were screened for their ability to induce TK at permissive and nonpermissive temperatures, and the effect of the extracellular calcium concentration on the induction of TK by serum and Ad5 was determined. Ad5-induced progression into S phase did not include many events which occur in G1 when the cells were stimulated by serum. Induction of TK by Ad5 and serum differed in sensitivity to AC, AM, and the calcium concentration

of the medium. Ad5 induction of TK was insensitive to AM and AD. Levels of TK induced by Ad5 and serum were additive indicating that separate mechanisms were involved. MGBG inhibited TK induction by both serum and Ad5 indicating that adenovirus infection does not override all of the normal cellular control mechanisms. Ad5 did not induce OD in G1-arrested cells. (56 Refs)

35. THE PLUS-STRAND LEADER RNA OF VSV INHIBITS DNA-DEPENDENT TRANSCRIPTION OF ADENOVIRUS AND SV40 GENES IN A SOLUBLE WHOLE-CELL EXTRACT.

McGowan JJ, Emerson SU, Wagner RR
Dept. Microbiology, Univ. Virginia, Sch. Medicine, Charlottesville, VA, 22908
Cell; 28(2):325-333 1982

In an attempt to determine the mechanism (or mechanisms) by which vesicular stomatitis virus (VSV) kills cells, products of VSV transcription were tested in a cell-free system for their capacity to inhibit transcription of SV40 DNA and plasmids containing adenovirus late promoter and adenovirus-associated RNA genes. VSV RNA transcripts and other RNAs were compared for their capacity to suppress transcription of these DNA templates by RNA polymerases and cofactors present in the HeLa-cell extract system. Relatively low concentrations of the plus-strand leader RNA made in vitro from the 3' end of the wild-type VSV genome were found to inhibit initiation of transcription catalyzed by both RNA polymerase II and RNA polymerase III. Polyadenylated VSV messengers and other natural and synthetic RNAs also caused some inhibitory effects on in vitro transcription from DNA templates, but only at extremely high concentrations. Compared with the wild-type plus-strand RNA leader, the leader RNA synthesized in vitro by defective-interfering VSV showed only limited capacity to inhibit RNA synthesis on adenovirus and SV40 DNA templates and only at concentrations at least 30 times greater than that of the wild-type leader. The existence of nucleotide sequences in wild-type leader RNA, not present in defective-interfering leader RNA, that could recognize and block promoters, polymerases or protein cofactors is discussed. (Author abstract) (36 Refs)

36. EXPRESSION OF HERPESVIRUS THYMIDINE KINASE GENE UNDER CONTROL OF EARLY PROMOTER OF SV40.

Pater MM, Pater A, Di Mayorca G, Smiley JR
Dept. Microbiology, New Jersey Medical Sch., Coll. Medicine and Dentistry New Jersey, 100 Bergen St., Newark, NJ, 07103
Virology; 117(2):536-540 1982

A 0.31-kilobase fragment of SV40 DNA containing the early promoter of this virus was isolated. The pX1 plasmid containing the herpesvirus thymidine kinase gene (TK) was cleaved with BglII and SalI to remove the TK promoter. The SV40 DNA fragment was mixed with the TK gene without its promoter and blunt-end ligated after treatment with S1 nuclease. The newly engineered plasmid was used for the transformation of TK-deficient LtA cells to TK+ phenotype. Blot hybridization experiments show that the plasmid is integrated into high-mol-wt DNA in both of the transformed colonies that we have studied. Also the mRNA in these cells is a hybrid molecule containing both TK and SV40 sequences. (Author abstract) (29 Refs)

37. IN VITRO FORMATION OF MULTIMERIC DNA STRUCTURES MEDIATED BY PURIFIED SIMIAN VIRUS 40 CHROMATIN.

Waldeck W, Zentgraf H, Sauer G
Inst. Virus Res., German Cancer Res. Center, 6900 Heidelberg, W. Germany
Proc Natl Acad Sci USA; 79(4,part1):1027-1031 1982

Simian virus 40 chromatin was incubated after purification by sucrose density-gradient centrifugation with various circular double-stranded DNA substrates. Monomeric rings were converted in the presence of Mg++ to structures possessing a higher degree of complexity. Dimeric catenanes, as well as multimeric linear structures and concatemers, were generated, indicating that recombination events had occurred in vitro involving covalent linkage between different DNA molecules. Furthermore, apparently fused dimeric rings were observed. Their structures suggest that they may be recombination intermediates such as those described in a prokaryotic system. Recombination did not take place between heterologous DNA substrates, as exclusively homologous multimeric DNA structures were observed. (Author abstract) (25 Refs)

38. EFFECT OF METHYLATION ON EXPRESSION OF MICROINJECTED GENES.

Waechter DE, Baserga R
Fels Res. Inst., Temple Univ. Sch. Medicine, Philadelphia, PA, 19140
Proc Natl Acad Sci USA; 79(4,part1):1106-1110 1982

The cloned genes for the simian virus 40 large tumor antigen and for herpes simplex virus (HSV) thymidine kinase (TK) were methylated with EcoRI methylase. The genes were microinjected into the nuclei of TK-deficient (tk-) cells, and expression of the genes was determined by immunofluorescence staining for the simian virus 40 large tumor antigen and by [3H]thymidine incorporation followed by autoradiography for HSV TK. We found that methylation of the simian virus 40 gene, under EcoRI or EcoRI* conditions, resulting in methylation at sites within the gene and in the surrounding sequences, has no effect on expression of the large tumor antigen when the gene is manually microinjected into mammalian nuclei. However, methylation of the HSV tk gene at the two EcoRI sites markedly reduces or abolishes the expression of this gene. Of the EcoRI sites of HSV tk is approx 1.1 kilobases downstream from the 3' end of the gene and is believed to have no regulatory function in the expression of the tk gene. The other EcoRI site is 79 base pairs upstream from the 5' end of the gene and has considerable homology to the regulatory sequence proposed by Benoist, O'Hare, Breathnach, and Chambon. Our results are direct proof that methylation can alter gene expression and also that the effect depends strictly on the sites that are methylated. (Author abstract) (40 Refs)

39. SIMIAN VIRUS 40 LARGE TUMOR ANTIGEN: A 'RNA BINDING PROTEIN'?

Khandjian EW, Loche M, Darlix JL, Cramer R, Turler H, Weil R
Dept. Molecular Biology, Univ. Geneva, 1211 Geneva, Switzerland
Proc Natl Acad Sci USA; 79(4,part1):1139-1143 1982

Simian virus 40 large tumor antigen was isolated by immunoaffinity chromatography from monkey or mouse cell cultures undergoing lytic or transforming infection. RNase-treated gel-purified large tumor antigen, on hydrolysis with alkali, gave about equimolar amounts of AMP, CMP, and UMP. Furthermore, RNA fragments of approx 45 nucleotides could be isolated from large tumor antigen purified by the same procedure.

Mapping of the T1 oligonucleotides showed a high complexity, as indicated by the presence o f unique sequences of 15-30 nucleotides and of poly(A). This is compatible with the hypothesis that these RNA fragments are derived from cellular pre-mRNAs or mRNAs. Our results suggest that Simian virus 40 large tumor antigen is a RNA-binding protein and might possibly be involved in regulation of synthesis, maturation, or translation of cellular mRNAs. (Author abstract) (39 Refs)

40. MUTATION CAUSING PREMATURE TERMINATION OF THE POLYOMA VIRUS MEDIUM T ANTIGEN BLOCKS CELL TRANSFORMATION.

Templeton D, Eckhart W
Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
J Virol; 41(3):1014-1024 1982

We used site-specific mutagenesis to introduce a termination codon, TGA, into the reading frame for the polyoma virus medium T antigen. We induced this mutation in a region of the polyoma genome in which the overlapping coding regions for the large and medium T antigens are translated in different reading frames. Therefore, the mutation terminated translation of the medium T antigen, but it caused only a single amino acid substitution in the large T antigen and did not affect the small T antigen. Cells infected by the mutant virus produced normal-size small and large T antigens. The infected cells produced a 28,000-dalton fragment of the 48,000-dalton medium T antigen, whose size and tryptic peptide map were consistent with its being a truncated N-terminal fragment terminating at the new termination codon of the mutant. Immunoprecipitates of mutant-infected cell extracts did not show medium-T-antigen-associated protein kinase activity. The mutant virus replicated normally in mouse 3T6 cells and induced cellular DNA synthesis in resting mouse 3T3 cells, but it failed to transform rat or hamster cells, as judged by focus formation and growth in agar. The mutant complemented a tsA mutant which affects the large T antigen for transformation, implying that the mutant defect for transformation was in the medium T antigen. These results imply that the small T antigen and the large T antigen together are insufficient to cause transformation and support the conclusion that the medium T antigen is essential for cell transformation by polyoma virus. (Author abstract) (31 Refs)

41. SITE-SPECIFIC CLEAVAGE/PACKAGING OF HERPES SIMPLES VIRUS DNA AND THE SELECTIVE MATURATION OF NUCLEOCAPSIDS CONTAINING FULL-LENGTH VIRAL DNA.

Vlazny DA, Kwong A, Frenkel N
(c/o Frenkel) Dept. Biology, Univ. Chicago, Chicago, IL, 60637
Proc Natl Acad Sci USA; 79(5):1423-1427 1982

Defective genomes present in serially passaged herpes simplex virus (HSV) stocks have been shown to consist of tandemly arranged repeat units containing limited sets of the standard virus DNA sequences. Invariably, the HSV defective genomes terminate with the right (S component) terminus of HSV DNA. Because the oligomeric forms can arise from a single repeat unit, it has been concluded that the defective genomes arise by a rolling circle mechanism of replication. We now report on our studies of defective genomes packaged in viral capsids accumulating in the nuclei and in mature virions (enveloped capsids) trans-located into the cytoplasm of cells infected with serially passaged virus. These studies have revealed that, upon electrophoresis in agarose gels, the defective genomes prepared from cytoplasmic virions comigrated with non-defective standard virus DNA (Mr 100 x 10(6)). In contrast, DNA prepared from capsids accumulating in nuclei consisted of both full-length defective virus DNA molecules and smaller DNA molecules of discrete sizes, ranging in Mr from 5.5 to 100 x 10(6). These smaller DNA species were shown to consist of different integral numbers (from 1 to approximately 18) of defective genome repeat units and to terminate with sequences corresponding to the right terminal sequences of HSV DNA. We conclude on the basis of these studies that (i) sequences from the right end of standard virus DNA contain a recognition signal for the cleavage and packaging of concatemeric viral DNA, (ii) the sequence-specific cleavage is either a prerequisite for or occurs during the entry of viral DNA into capsid structures, and (iii) DNA molecules significantly shorter than full-length standard viral DNA can become encapsidated within nuclear capsids provided they contain the cleavage/packaging signal. However, capsids containing DNA molecules significantly shorter than standard virus DNA are not translocated into the cytoplasm. (Author abstract) (31 Refs)

42. COMPARISON OF THYMIDINE KINASE ACTIVITIES INDUCED IN CELLS PRODUCTIVELY INFECTED WITH HERPESVIRUS SAIMIRI AND HERPES SIMPLEX VIRUS.

Honess RW, O'Hare P, Young D
Div. Virology, Natl. Inst. Medical Res., Mill Hill, London NW7 1AA, England
J Gen Virol; 58(part2):237-249 1982

The replication of unselected strains of herpesvirus saimiri (HVS) was sensitive to bromodeoxyuridine and bromovinyldeoxyuridine (BVdU) but insensitive to acycloguanosine (ACG), in contrast to the growth of herpes simplex virus (HSV) which was sensitive to all three analogues. Mutants of HVS resistant to bromodeoxyuridine and BVdU could be selected by growth in the presence of these inhibitors. Productive infections of owl monkey kidney or Vero cell cultures by unselected strains of HVS resulted in increases in a thymidine kinase (TK) activity which was deficient in cells infected with bromodeoxyuridine-resistant mutants of the virus. Induction of the virus enzyme promoted a net increase in the uptake and incorporation of exogenous labeled thymidine in the face of the progressive inhibition of the overall incorporation of [35S]methionine and [3H]uridine into productively infected cells. The TK induced in cells infected w ith HVS differed from the major activity of uninfected cells and resembled that encoded by HSV in its capacity to phosphorylate iododeoxyuridine and in the sensitivity of all the thymidine phosphorylating activity to competition by BVdU. However, in contrast to the HSV Tk, which phosphorylated deoxycytidine and iododeoxycytidine relatively efficiently and was sensitive to ACG, the HVS enzyme did not phosphorylate deoxycytidine or iododeoxycytidine and was insensitive to ACG. Whilst HVS, therefore, shares the characteristic of other members of the herpesvirus group of inducing a novel TK, the properties of the HVS-induced enzyme differ significantly from the enzyme of the prototype herpesvirus, HSV. The properties of the HVS TK are nonetheless sufficiently distinct from those of the uninfected cell to provide a possible basis for selective antiviral chemotherapy based on preferential phosphorylation of nucleoside analogues such as BVdU by infected cells. (Author abstract) (40 Refs)

43. 5-AZACYTIDINE-INDUCED REACTIVATION OF A HERPES SIMPLEX THYMIDINE KINASE GENE.

Clough DW, Kunkel LM, Davidson RL
Center for Genetics, Univ. Illinois Medical Center, 808 South Wood St., Chicago, IL, 60612
Science; 216(4541):70-73 1982

Mouse cells transformed with herpes simplex virus and containing the viral thymidine kinase (TK) gene in an inactive state were treated with 5-azacytidine. The result was the reexpression of the viral TK gene. Two days of exposure to 5-azacytidine followed by 2 days of expression time was sufficient for maximal induction of the TK+ phenotype. The induction of TK expression by 5-azacytidine was concentration-dependent, with maximal induction at 10 micromoles per liter. 5-Azacytidine also inhibited the decay of TK expression in TK+ transformants removed from selective conditions. Analysis of the methylation patterns of the viral TK gene with restriction endonucleases Hpa II and Msp I showed the active gene to be unmethylated, the inactive gene methylated, and the 5-azacytidine-induced gene unmethylated. (Author abstract) (18 Refs)

44. INHIBITION OF HERPES SIMPLEX VIRUS REPLICATION BY SUCCINYL CONCANAVALIN A.

Garrity P, Szelc C, Paquette C, McEvoy M, Millette R, Adler R
Dept. Natural Sciences, Univ. Michigan-Dearborn, Dearborn, MI, 48128
Antimicrob Agents Chemother; 21(3):450-455 1982

Incubation of herpes simplex virus Type 1-infected cells with succinyl-concanavalin A, a derivative of the jack bean lectin concanavalin A, resulted in the decreased production of virus. The mode of inhibition by the lectin was unclear. No effect was apparent on the level of viral DNA synthesis. However, incubation of infected cells with increasing concentrations of the lectin appeared to result in a decrease in the quantity of viral protein produced within the cell. A reduction in the virus titer of 57 to 64% was observed upon direct incubation of extracellular virus in the presence of 50 to 100 ug of succinyl-concanavalin A per ml. (Author abstract) (35 Refs)

45. EVIDENCE AGAINST INVOLVEMENT OF HOST TRANSCRIPTION IN THE REPLICATION OF VACCINIA AND HERPES SIMPLEX VIRUSES.

Silver M, Dales S
(c/o Dales) Cytotobiology Group, Dept. Microbiology and Immunology, Univ. Western Ontario, London, Ontario N6A 5C1, Canada
Virology; 118(1):214-218 1982

Vaccinia and herpes simplex viruses are unable to multiply in cells treated with the toxin alpha-amanitin, which specifically blocks host transcription involving RNA polymerase II (pol II). However, these agents are replicated in host cells made nonfunctional by intense gamma irradiation when RNA synthesis, including poly(A)-containing RNA and induction of biological functions, exemplified by the antiviral state, are inhibited. From this and previous evidence it is concluded that requirement for pol II or subunit(s) thereof by pox- and herpesviruses is probably related to transcription from the viral, not the host genome. (Author abstract) (11 Refs)

46. EPSTEIN-BARR VIRUS RECEPTOR EXPRESSION IS CORRELATED TO CELL CYCLE PHASE.

Wells A, Steen HB, Godal T, Klein G
Box G, Brown Univ. Sch. Medicine, Providence, RI, 02912
J Recept Res; 2(3):285-298 1981

Fluorescent isothiocyanate (FITC)- conjugated virions was directly absorbed by Epstein-Barr virus (EBV), incubated with Raji, Daudi, or YAC cell lines, and for DNA content determination were stained with propidium iodide. FITC binding and labeled DNA were simultaneously measured by flow cytometry. EBV receptor was present throughout the whole cell cycle, but its level of expression increased significantly in both G1 and G2/M phases, but not in S phase. The increase in G1 was greater than in the other phases. The receptor level on the live cells increased directly with cell size; the rate of increase, determined as the receptor density, was inversely correlated with cell size. Early in G1 cells seem to synthesize rapidly the EBV receptor to increase the structure's density. This observation may have in vivo implications in relation to the timing of synthesis after initial cell stimulation. (29 Refs)

47. DNA SEQUENCE OF THE 5' TERMINUS CONTAINING THE REPLICATION ORIGIN OF PARVOVIRUS REPLICATIVE FORM DNA.

Rhode SL, Klaassen B
Inst. Medical Res. Bennington, Bennington, VT, 05201
J Virol; 41(3):990-999 1982

The nucleotide sequence of the 5' terminus of the parvovirus H-1 was determined. There are two orientations of the 242-base-pair terminal palindrome in native replicative form DNA, one inverted with respect to the other. Adjacent to the terminal palindrome is an AT-rich region that is noncoding and contains a 55-base-pair tandem repeat. The addition mutant of H-1, DI-1, was also sequenced in this regi on and shown to have three copies of the tandem repeat sequence. Similarly, the related parvovirus H-3 contains only one copy of this repeat sequence. This region contains the replication origin for parvovirus replicative form DNA replication. Some of the implications of these results are discussed. (Author abstract) (37 Refs)

48. DNA POLYMERASE REQUIREMENTS FOR PARVOVIRUS H-1 DNA REPLICATION IN VITRO.

Kollek R, Tseng BY, Goulian M
Institut fur Biochemie, Univ. Munchen, Karlstrasse 23, D-8000 Munchen 2, W. Germany
J Virol; 41(3):982-989 1982

An in vitro system using nuclei from parvovirus H-1-infected cells was used to characterize the influence of inhibitors of mammalian DNA polymerases on viral DNA synthesis. The experiments tested the effects of aphidicolin, which is highly specific for DNA polymerase alpha, and 2',3'-dideoxythymidine-5'-triphosphate (ddTTP), which inhibits cellular DNA polymerase in the order gamma greater than beta greater than alpha. Both aphidicolin and ddTTP were inhibitory, indicating that both polymerase alpha and a ddTTP-sensitive enzyme are required for viral DNA synthesis. This was seen more clearly in kinetic measurements, which indicated an initial period of rapid DNA synthesis with the participation of polymerase alpha, followed by a period of less rapid, but more sustained, rate of DNA synthesis carried out by a ddTTP-sensitive enzyme, probably polymerase gamma. One interpretation of the results is that polymerase alpha function in a strand displacement stage of the viral DNA replication mechanism,

whereas polymerase gamma serves to convert the displaced single strands back to double-strand replicative form. (Author abstract) (42 Refs)

VIRAL GENETICS

49. MUTATIONS IN THE HERPES SIMPLEX VIRUS DNA POLYMERASE GENE CAN CONFER RESISTANCE TO 9-BETA-D-ARABINOFURANOSYLADENINE.

Coen DM, Furman PA, Gelep PT, Schaffer PA
Dept. Microbiology and Molecular Genetics, Sidney Farber Cancer Inst., Harvard Medical Sch., Boston, MA, 02115
J Virol; 41(3):909-918 1982

Mutants of herpes simplex virus type 1 resistant to the antiviral drug 9-beta-D-arabinofuranosyladenine (araA) have been isolated and characterized. Ara-A-resistant mutants can be isolated readily and appear at an appreciable frequency in low-passage stocks of wild-type virus. Of 13 newly isolated mutants, at least 11 were also resistant to phosphonoacetic acid (PAA). Of four previously described PAA-resistant mutants, two exhibited substantial araA resistance. The araA resistance phenotype of one of these mutants, PAAr5, has been mapped to the HpaI-B fragment of herpes simplex virus DNA by marker transfer, and araA resistance behaved in marker transfer experiments as if it were closely linked to PAA resistance, a recognized marker for the viral DNA polymerase locus. PAAr5 induced viral DNA polymerase activity which was much less susceptible to inhibition by the triphosphate derivative of araA than was wild-type DNA polymerase. These genetic and biochemical data indicate that the herpes simplex virus DNA polymerase gene is a locus which, when mutated, can confer resistance to araA and thus that the herpes simplex virus DNA polymerase is a target for this antiviral drug. (Author abstract) (41 Refs)

IMMUNOLOGICAL STUDIES

50. ACTIVATION OF NATURAL KILLER CELLS AND INDUCTION OF INTERFERON AFTER INJECTION OF MOUSE HEPATITIS VIRUS TYPE 3 IN MICE.

Schindler L, Engler H, Kirchner H
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Infect Immun; 35(3):869-873 1982

High levels of natural killer (NK) cell activity and high titers of interferon were observed in the peritoneal exudate of C57BL/6 mice 20 to 50 h after injection of mouse hepatitis virus Type 3 (MHV3) but not during the first 10 h after infection. C57BL/6 mice were susceptible to MHV3 infection and showed high titers of MHV3 in the peritoneal exudate 48 h after infection. A/J mice, in contrast, were resistant to the dose that killed 100% of the C57BL/6 mice (10 macrophage-infecting doses) and showed considerably lower virus titers than those shown by C57BL/6 mice. NK cell activity and interferon titers were significantly lower in the peritoneal exudate of A/J mice than in that of C57BL/6 mice. Serum interferon titers were also lower in A/J mice. Thus, our data show an inverse relationship between resistance and the levels of these two parameters. The data suggest that, in contrast to the situation observed with certain herpesviruses, interferon and NK cells may not be of overwhelming importance in the defense of mice against MNV3. (Author abstract) (14 Refs)

51. TWO DISTINCT HUMAN CLONED T CELL LINES THAT EXHIBIT NATURAL KILLER-LIKE AND ANTI-HUMAN EFFECTOR ACTIVITIES.

Sugamura K, Tanaka Y, Hinuma Y
Inst. Virus Res., Kyoto Univ., Sakyo-ku, Kyoto 606, Japan
J Immunol; 128(4):1749-1752 1982

Cloned T cell lines from mixed lymphocyte cultures stimulated with autologous Epstein Barr virus- (EBV) transformed lymphoblastoid cell line (LCL) cells were established using a limiting dilution technique in the presence of T cell growth factor (TCGF). The T cell lines included two distinct clones of cytotoxicity T cells (Tc) in addition to EBV-specific Tc. A cytotoxic profile of one cloned line was similar to that of endogenous NK cells in peripheral blood. The other cloned Tc line showed an anti-human cytotoxicity. The susceptible targets for this latter Tc line were various human cells, including autologous LCL and peripheral blood lymphocytes (PBL), stimulated with pokeweed mitogen, along with NK-sensitive and NK-resistant cell lines. Weak cytotoxic activity was detected against various xenogeneic cell line s. Furthermore, autologous and allogeneic cloned T cell lines were resistant to killing by the anti-human effector clone. These two distinct cloned Tc lines expressed the Leu-1 and Leu-2a antigens, which are markers of suppressor/cytotoxic T cells. (Author abstract) (23 Refs)

52. EPSTEIN-BARR VIRUS (EBV) RECEPTOR IMPLANTATION ONTO HUMAN B LYMPHOCYTES CHANGES IMMUNOGLOBULIN SECRETION PATTERNS INDUCED BY EBV INFECTION.

Tsukuda K, Volsky DJ, Shapiro IM, Klein G
Dept. Tumor Biology, Karolinska Institutet, S-104 01 Stockholm, Sweden
Eur J Immunol; 12(1):87-90 1982

Mosaic membrane vesicles containing both Epstein-Barr virus (EBV) receptors and Sendai virus envelope proteins were allowed to form by the previously described membrane solubilization and co-reconstitution technique. The vesicles were allowed to fuse with the membranes of normal human B lymphocytes, whereafter the cells were infected with transforming EBV (B95-8 substrain). Compared to similarly infected but otherwise unmanipulated cells, the receptor-implanted lymphocytes responded with a larger number of EBV-determined nuclear antigen positive and immunoglobulin-secreting plaque-forming cells (PFC). Moreover, there was a clear increase of the IgG/IgM PFC ratio in the receptor-implanted B lymphocytes. These results show that not all human B lymphocytes that can potentially be activated by EBV express functional EBV receptors. B lymphocytes programmed to secrete IgG appear to be more defective in this respect than IgM secretors. (Author abstract) (27 Refs)

53. A MARMOSET T LYMPHOMA WHICH FUNCTIONS AS A HUMAN AMPLIFIER T CELL.

Marchalonis JJ, Strelkauskas AJ
Dept. Biochemistry, Medical Univ. South Carolina, 171 Ashley Ave., Charleston, SC, 29425
J Immunogenet; 8(6):477-483 1981

A long-term in vitro grown T-cell line derived from the cotton-topped marmoset (Saguinus oedipus) infected with

Herpesvirus saimiri is reported, which possessed the surface properties and functional characteristics of a human helper T cell. A rabbit antiserum to the cell line also identified a subset of human peripheral T cell that has the phenotype of helper T cells. Direct addition of the marmoset line to purified normal human B cells amplified the capacity of the B cells to secrete immunoglobulin as defined by the reverse hemolytic plaque assay. This demonstration of collaboration between a New World monkey-derived T-cell line and human B-cells in elaboration of antibody secretion is the first such example of functional cooperation of T and B cells across such a large phylogenetic barrier. (12 Refs)

54. SPECIFICITY AND ROLE OF ANTIBODIES TO TUMOR ASSOCIATED ANTIGENS: ONE VIEW FROM THE LABYRINTH.

Klein PA
Tumor Biology Unit, Dept. Pathology, Coll. Medicine, Univ. Florida, Gainesville, FL, 32610
The Handbook of Cancer Immunology: Volume 9- Humoral Immunity in Relation to Cancer. Waters H, ed. New York, Garland STPM Press, 289 pp., 1981.

Animals bearing some types of tumors develop antibodies in response to tumor-associated antigens (TAAs). The net results of the host-tumor-TAA interaction range from inhibition to promotion of local tumor growth and metastatic spread. The natures of these TAAs are poorly understood. Cell-mediated mechanisms depend on T-lymphocytes are important in host reactions to immunogenic transplantable tumors. Only highly immunogenic tumors induced by certain DNA viruses elicit immune surveillance reactions Because of the major differences between transplantable animal tumors and spontaneous human tumors, the applicability of these studies on tumor immunology to clinical research is not clear. (91 Refs)

55. INDUCTION AND INHIBITION OF THE IMMUNE RESPONSE TO TUMORS INDUCED BY SIMIAN VIRUS 40 (SV40).

Blasecki JW
Pharmaceuticals Div., Stine Lab., E.I. du Pont de Nemours and Co., Newark, DE, 19711
The Handbook of Cancer Immunology: Volume 8- Tumor Antigens: Structure and Function. Waters H, ed. New York, STPM Garland Press, 418 pp., 1981.

The growth or rejection of SV40-induced tumors in immunocompetent hosts is determined by a dynamic balance among cellular and humoral responses to the tumor and the effects of products shed from the tumor cells (apparently surface-associated antigens) on this balance. The first step in the immune response to the tumor seems to be the processing of SV40 tumor-specific transplantation antigens (TSTA) by the macrophages (or perhaps natural killer cells) and the presentation of TSTA to the T-lymphocytes. This leads to the appearance of sensitized T-cells having specific cytolytic activity against SV40 tumor cells. Cytotoxic antibodies apparently do not play a significant role in tumor rejection. The induction of immunity to SV40 TSTA is not the only aspect of the host-tumor interaction, but inhibition of the antitumor immune response is apparently critical to favoring tumor growth. SV40 TSTA seems to play an important role in abrogating specific cellular immunity to SV40 tumors in vivo. Antibody or antigen-antibody complexes seem to block cellular immunity to SV40 tumors at the cellular level. Progressive tumor growth apparently causes a specific or nonspecific suppression of helper T-cell activity. The suppression of antibody synthesis in tumor-bearing hosts may be involved in preventing antibody-mediated sequestration of tumor-associated antigens, thus enhancing tumor growth. Soluble blocking factors and suppressor cells also seem to be involved in regulating host immune responses to SV40-induced tumors. (217 Refs)

56. HETEROGENEITY OF ALLOGENEIC RESTRICTION OF HUMAN CYTOTOXIC T CELL CLONES SPECIFIC FOR EPSTEIN BARR VIRUS.

Tanaka Y, Sugamura K, Hinuma Y
Inst. Virus Res., Kyoto Univ., Sakyo-ku, 606, Japan
J Immunol; 128(3):1241-1245 1982

Clones of human cytotoxic T cells (Tc) specific for Epstein Barr virus (EBV) were isolated from peripheral blood lymphocyte (PBL) cultures stimulated repeatedly with autologous EBV-transformed lymphoblastoid cell line (LCL) cells in vitro. The method employed to clone EBV-specific Tc was a limiting dilution technique utilizing T cell growth factor (TCGF). The EBV specificity of Tc clones was determined by showing that they were significantly cytotoxic for autologous LCL cells but not for either autologous PBL or (natural killer-sensitive) K-562 cells. Eight EBV-specific Tc clones derived from a single donor exhibited distinct cytotoxic patterns against allogeneic LCL targets. Two clones were cytotoxic to LCL targets sharing both HLA-A26 and B15 antigens with effectors, and killing by two other clones was strongly restricted to autologous LCL cells. The four remaining clones showed cytotoxicities against various allogeneic LCL targets irrespective of HLA antigen expression. Eight EBV-specific Tc clones derived from a second donor also exhibited a wide spectrum of cytotoxicity to allogeneic LCL targets. We conclude that EBV-specific Tc, induced in vitro, consist of a number of clones with respect to restrictions imposed by the major histocompatibility complex. The determinants regulating these restrictions may include not only private HLA antigenic determinants that are defined by the HLA serotyping, but also undefined HLA antigenic determinants. (Author abstract) (30 Refs)

57. ACTIVATION OF SUPPRESSOR T CELLS BY AUTOLOGOUS LYMPHOBLASTOID CELLS: A MECHANISM FOR FEEDBACK REGULATION OF IMMUNOGLOBULIN SYNTHESIS.

James SP, Yenokida GG, Graeff AS, Strober W
Immunophysiology Section, Metabolism Branch, NCI, NIH, Bethesda, MD, 20205
J Immunol; 128(3):1149-1154 1982

In this study, the regulatory potential of T cells activated as a result of exposure to autologous lymphoblastoid cells was examined. In initial studies it was observed that the proliferative response of T cells induced by exposure [illegible] formed or Epstein Barr virus [illegible] transformed autologous lymphoblastoid cells was greater than the proliferative response of T cells induced by exposure to unstimulated autologous B cells. Such activated T cells profoundly suppressed pokeweed mitogen (PWM)-stimulated immunoglobulin synthesis by autologous indicator cells, but compared to fresh T cells or cultured T cells, these activated cells had a diminished capacity to provide help for PWM-stimulated immunoglobulin synthesis. In additional studies it was found that (1) both PWM-transformed B cells and EBV-transformed B cells were able to activate suppressor T cells, suggesting that the mechanism of activation is independent of the agent used to transform the lymphoid cells; (2) T cells cultured with PWM-induced lymphoblastoid cells did not suppress immunoglobulin synthesis when added to PWM-stimulated cells after 3 days of

culture, indicating that suppression was not due to killing of immunoglobulin-secreting cells; (3) mitogen-induced lymphoblastoid cells acquired maximal ability to activate suppressor T cells after 3 days of culture with the mitogen, indicating that lymphoid cells had to undergo mitogen-induced changes to activate suppressor T cells. These results are compatible with the concept that when B cells are activated to differentiate into immunoglobulin-secreting cells, they express new determinants that are capable of activating suppressor T cells, which can then act to inhibit further B cell differentiation. (Author abstract) (26 Refs)

58. PROTECTION OF NEWBORN MICE FROM A LETHAL HERPES SIMPLEX VIRUS INFECTION BY HUMAN INTERFERON, ANTIBODY, AND LEUKOCYTES.

Kohl S, Loo LS, Greenberg SB
Program Infectious Diseases, Univ. Texas Medical Sch., P.O. Box 20708, Houston, TX, 77025
J Immunol; 128(3):1107-1111 1982

Newborn mice infected ip with a lethal dose of herpes simplex virus (HSV) were protected by a combination of human interferon and human mononuclear cells (MC) when it was administered ip 1 day before infection (35.2% survived, p less than 0.001). The HSV immune status of the MC donors did not affect this protection. The number of MC required in the presence of 100 units of interferon was 5 x 10(6) to 5 x 10(5)/mouse. The dose of interferon required in the presence of 5 x 10(6) MC was 100 to 10 U. Highest survival was achieved with 5 x 10(6) MC and 100 U of interferon. The active human effector cell in this system was either a lymphocyte or a monocyte-macrophage but not a polymorphonuclear leukocyte. Treatment of mice with interferon and MC after viral inoculation was not successful. Protection by human WISH cells (a non-leukocyte) and interferon was demonstrated (55% survival, p less than 0.001), which suggested that protection was due to interferon induced cross-species active antiviral protein production. The human interferon stimulated human but not murine or WISH cell natural killer cytotoxicity (NKC) in vitro. Mice receiving human interferon, MC, and subneutralizing doses of anti-HSV antibody before HSV infection were almost completely (95.2%) protected, and were protected significantly better (p less than 0.001) than mice receiving either MC plus interferon or MC plus antibody. Although adult MC could mediate this protection, all mice receiving MC from neonatal humans plus antibody and interferon died (p less than 0.001). In vitro experiments showed that protection was not due to viral neutralization. This is the first evidence for either in vivo human antiviral NKC or cross-species antiviral protein protection as well as defective in vivo human neonatal MC function in this system. (Author abstract) (29 Refs)

OTHER RELATED STUDIES

59. MOLECULAR CLONING AND PARTIAL NUCLEOTIDE SEQUENCE OF HUMAN PAPILLOMAVIRUS TYPE 1A DNA.

Clad A, Gissmann L, Meier B, Freese UK, Schwarz E
(c/o Schwarz) Institut fur Virologie, Zentrum fur Hygiene, Universitat Freiburg, Hermann-Herder-Strasse 11, 7800 Freiburg, W. Germany
Virology; 118(1):254-259 1982

Papilloma viruses contain a DNA genome of about 5 x 10(6). They belong in the papovavirus group and induce epithelial proliferation in a variety of animal species and in man. To date, eight types of papillomaviruses have been identified in different human papillomas, some of which show an increased tendency for malignant conversion. Due to the inability to propagate papillomaviruses in tissue culture, almost nothing is known about their genetic organization and gene expression. The use of recombinant DNA techniques now renders it possible to study in detail the molecular biology of papillomaviruses. Here we report the molecular cloning of the DNA of human papillomavirus Type 1a (HPV1a) in Escherichia coli using pBR 322 as vector and the nucleotide sequence of part of the HPV1a genome. The region of 1819 base pairs contains one of two pairs of short inverted repetitive sequences detected earlier by electron microscopic studies. Within the DNA sequence, three potential coding regions of only one orientation have been observed which allows prediction of the direction of transcription in this part of the HPV1a genome. (Author abstract) (22 Refs)

60. TRANSFER OF GENES TO CHINESE HAMSTER OVARY CELLS BY DNA-MEDIATED TRANSFORMATION.

Abraham I, Tyagi JS, Gottesman MM
Lab. Molecular Biology, NCI, Bethesda, MD, 20205
Somatic Cell Genet; 8(1):23-39 1982

Successful transfer of the thymidine kinase gene of herpes simplex virus 1 (HSV-tk) to Chinese hamster ovary (CHO) cells is reported. Maximal transformation frequencies of 5 x 10(-5) to 1 x 10(-4) were obtained. Transformation of the cells was associated with a high incidence of cotransformation of unselected sequences; transformants derived from HSV-tk DNA transfer were relatively stable in retaining the selected phenotype in nonselective medium. (41 Refs)

61. ANTIBODIES CONJUGATED TO POTENT CYTOTOXINS AS SPECIFIC ANTITUMOR AGENTS.

Moolten FL, Schreiber BM, Zajdel SH
Boston Univ. Sch. Medicine, Boston, MA
Immunol Rev; 62:47-73 1982

The ability of toxins, enzymes, radioisotopes, and cytotoxic drugs linked to antibodies to selectively kill cells bearing surface antigens that are targets for the antibodies is reviewed. Criteria for the complexes include the following: the cytotoxin must be capable of acting after delivery to the cell surface by the antibody, the desired biologic properties of the components must survive the conjugation process, the conjugate must be stable until delivered to its site of action, the specificity of the conjugate must reflect primarily the specificity of the antibody complex, and the toxic moiety must be extraordinarily potent. Conjugates tested for antitumor activity include diphtheria toxin conjugates, Pseudomonas aeruginosa toxin conjugates, ricin conjugates, and phospholipase and ribonuclease conjugates. In vitro, each of the agents, when conjugated to antibodies directed against a variety of cell-surface antigens, was selectively cytotoxic to cells bearing those antigens. In vivo, diphtheria toxin conjugated to antibodies directed against the dinitrophenol (DNP) hapten reduced the incidence and growth rate of tumors in hamsters concurrently inoculated with DNP-coated sarcoma cells. When tested against a more sensitive Simian virus 40-induced tumor, two conjugate preparations induced permanent regressions of established tumor in 20% and 56% of treated hamsters. (65 Refs)

62. LACK OF EFFICACY OF 2-DEOXY-D-GLUCOSE IN THE TREATMENT OF EXPERIMENTAL HERPES GENITALIS IN GUINEA PIGS.

Shannon WM, Arnett G, Drennen DJ
Kettering-Meyer Lab., Southern Res. Inst., Birmingham, AL, 35255
Antimicrob Agents Chemother; 21(3):513-515 1982

Topical treatment of herpes genitalis in female guinea pigs with 2-deoxy-D-glucose in either agarose gels or miconazole nitrate ointment failed to prevent the development of genital lesions or to reduce the mean titers of recoverable virus in vaginal swabs from infected animals. In contrast, phosphonoacetic acid was therapeutically effective. (Author abstract) (15 Refs)

63. TOPICAL POLYRIBOINOSINIC-POLYRIBOCYTIDYLIC ACID COMPLEX IN THE TREATMENT OF RECURRENT GENITAL HERPES.

Crane LR, Levy HB, Lerner AM
(c/o Lerner), Hutzel Hosp. Medical Unit, Detroit, MI, 48201
Antimicrob Agents Chemother; 21(3):481-485 1982

Polyriboinosinic-polyribocytidylic acid complexed with poly-l-lysine and carboxymethylcellulose [poly(ICLC)] is a potent interferon inducer when given parenterally to humans. Topical application in animal models has shown beneficial antiviral and clinical effects. In a randomized, double-blinded, placebo-controlled trial of topical poly(ICLC) in recurrent genital herpes simplex virus infection, five clinical and two virological parameters were followed. Fifty-seven men and women, with 78 recurrences of genital herpes, were stratified by sex. No clinical or antiviral differences between poly(ICLC) and placebo groups in either stratum were found. Further analysis of male subgroups by age and size of lesions showed no changes in the rapidity of healing or viral shedding. (Author abstract) (7 Refs)

64. PHARMACOKINETICS AND TOLERANCE OF ACYCLOVIR, A NEW ANTI-HERPESVIRUS AGENT, IN HU MANS.

Laskin OL, Longstreth JA, Saral R, De Miranda P, Keeney R, Lietman PS
Div. Clinical Pharmacology, Johns Hopkins Univ., Baltimore, MD, 21205
Antimicrob Agents Chemother; 21(3):393-398 1982

The pharmacokinetics and tolerance of acyclovir administered intravenously in single doses of 2.5, 5.0, 10.0 and 15.0 mg/kg were studied in 13 volunteers. The mean concentrations (+- standard deviations) at the end of infusion as measured by radioimmunoassay were 4.52 +-0.31, 8.28 +-2.61, 14.6 +-2.30 and 22.7 +-10.4 ug/ml, respectively. Drug elimination during and after the infusion of acyclovir was well described by a two-compartment open model. The mean terminal plasma half-life for each of the groups was 2.85, 2.80, 3.30, and 2.38 hr, respectively. Within 72 hr after the start of the infusion, 70% of the administered drug was recovered in the urine as unchanged acyclovir. The renal clearance of acyclovir accounted for about 77% of the total clearance and was about threefold greater than the creatinine clearance. This confirms that acyclovir is eliminated predominantly by the kidneys in patients with normal renal function and suggests that renal secretion and glomerular filtration may both be involved. The only adverse effect found by clinical and laboratory monitoring was irritation at the iv site after extravasation (in 2 cases), which resolved without significant sequelae. (Author abstract) (11 Refs)

65. MICROINJECTION OF NUCLEIC ACIDS INTO CULTURED MAMMALIAN CELLS BY ELECTROPHORESIS.

Ocho M, Nakai S, Tasaka K, Watanabe S, Oda T
Dept. Biochemistry, Cancer Inst., Okayama Univ. Medical Sch., Okayama 700, Japan
Acta Med Okayama; 35(5):381-384 1981

Simian virus 40 (SV40) DNA was microinjected into cultured mammalian cells by means of electrophoresis (iontophoresis). Successful transfer of DNA into cells was confirmed by detecting SV40 T antigen using the indirect immunofluorescent technique. (Author abstract) (6 Refs)

66. TUMOUR-PROMOTING DITERPENE ESTERS PREVENT MACROPHAGE ACTIVATION.

Keller R, Keist R, Hecker E
Immunobiology Res. Group, Inst. Immunology and Virology, Univ. Zurich, 8032 Zurich, Switzerland
Br J Cancer; 45(1):144-146 1982

Peritoneal wash-outs from normal DA rats or C3H/J mice were interacted for 8 hr with macrophage-activating lymphokines. 14C-Deoxythymidine-labeled DA rat polyoma-induced tumor cells were interacted with effector cells for 36 hr at 37 C; the released radioactivity was measured and net cytotoxicity calculated. The phorbol esters 12-O-tetradecanoyl-phorbol-13-acetate (TPA), phorbol-12,13-didecanoate (PDD), and the 1alpha-alkyldaphnane, Pimelea factor P2, were similarly active in preventing lymphokine-induced or spontaneous enhancement of macrophage tumoricidal activity. Manifestation of macrophage cytotoxicity was inhibited whether or not effector cells were first pretreated for 4 hr with tumor promoters and the washed effector cells interacted with prelabeled targets, or the promoting agents were present throughout the entire 36-hr effector/target cell interaction. Thus, active tumor promoters of the phorbol diester- and daphnane-type prevent lymphokine-induced enhancement of macrophage cytolysis; such a capacity to suppress activation or manifestation of cytotoxicity by rat and mouse macrophages may be common to all tumor promoters. Tumor promoters may facilitate the outgrowth of transformed cells by stimulation of their multiplication and functional capacities and by suppression of the host's cellular antitumor effector systems. (11 Refs)

67. GENE SHUTTLING: MOVING OF CLONED DNA INTO AND OUT OF EUKARYOTIC CELLS.

Lindenmaier W, Hauser H, De Wilke IG, Schutz G
Gesellschaft fur Biotechnologische Forschung mbH, Braunschweig, W. Germany
Nucleic Acids Res; 10(4):1243-1256 1982

Successful shuttling of cloned DNA in eukaryotic cells should allow isolation of expressed genes. We tested the utility of cosmids for moving DNA into and out of eukaryotic cells. The unique cleavage of DNA at the cos site by the terminase function of lambda was exploited to maintain the linkage between the vector and inserted gene sequences, a prerequisite for successful rescue of the transforming DNA from high mol wt DNA of the eukaryotic transformant. A cosmid recombinant containing the HSV thymidine kinase gene and a lambda recombinant containing the chicken thymidine kinase gene were used to test the feasibility of this method. it was found that these recombinants can be rescued with high efficiency from DNA of HAT-resistant cells. (Author abstract) (18 Refs)

68. FELINE LEUKEMIA VIRUS AND HEPATITIS B VIRUS: A COMPARISON OF LATE MANIFESTATIONS.

Francis DP, Essex M, Maynard JE
Hepatitis Lab., Bureau Epidemiology, Center for Disease Control, 4402 North 7th St., Phoenix, AZ, 85014
Prog Med Virol; 27:127-132 1981

Similarities and differences between feline leukemia virus (FeLV) and hepatitis B virus (HBV) are reviewed, and estimates of outcomes of HBV infection are made in a manner similar to that used with FeLV from infection-rate and mortality statistics. Cancer is probably a rare manifestation of HBV infection in the United States (0.4% of infections), whereas deaths from cirrhosis are a much more common outcome (1.8%). HBV fits a pattern expected for a naturally-occurring oncogenic virus, and treatment and research should be geared to such a hypothesis. (15 Refs)

69. CYTOGENETIC EFFECTS OF CHANEROL.

Sokova OI, Volgareva GM, Kulagina OE, Kopnin BP, Pogosiants EE
All-Union Cancer Res. Center, Moscow, USSR
Eksp Onkol; 4(1):42-46 1982

Cytogenetic effects of the antineoplastic agent chanerol were studied in culture d human peripheral blood lymphocytes, human melanoma MeWo line, primary culture of hamster embryonal fibroblasts, and hamster embryonal fibroblasts transformed with Simian virus SV-40. Statistically significant increase of the incidence of chromosome aberrations was detected only in SV-40-transformed fibroblasts. Chanerol did not increase the frequency of sister chromatid exchanges. Mutagenic effect of chanerol depended upon the duration of exposure. (17 Refs) (In RUS)

70. RETROVIRUS LONG TERMINAL REPEATS ACTIVATE EXPRESSION OF CODING SEQUENCES FOR THE HERPES SIMPLEX VIRUS THYMIDINE KINASE GENE.

Joyner A, Yamamoto Y, Bernstein A
Ontario Cancer Inst., Univ. Toronto, 500 Sherbourne St., Toronto, Ontario, M4X 1K9, Canada
Proc Natl Acad Sci USA; 79(5):1573-1577 1982

In this study, we demonstrate that the long terminal repeats (LTRs) of a murine retrovirus can activate expression of heterologous gene coding sequences from which a functional promoter region has been deleted. Recombinant plasmid clones were obtained that contained both cloned fragments of Friend spleen focus-forming virus (SFFV) DNA and the herpes simplex virus (HSV) thymidine kinase (TK; ATP:thymidine 5'-phosphotransferase, EC 2.7.1.21) gene (tk). The effects of the LTR on tk expression were determined by constructing clones containing tk coding sequences with or without 5' sequences necessary for the initiation of transcription, inserted either 200 or 1,200 base pairs downstream from the SFFV 5' LTR. The expression of the HSV TK protein by these clones was tested by gene transfer of the clones into TK- mouse cells and assay of TK enzyme activity in TK+ transformants. These experiments demonstrate that: (i) the SFFV 5' LTR activates expression of tk coding sequences when these sequences are inserted 200 base pairs downstream from, and in the same orientation as, the LTR; (ii) tk is not activated when placed 1,200 base pairs downstream from, and in the same orientation as, the LTR or when tk is inserted in either site in the opposite orientation as the LTR; (iii) the SFFV 5' LTR does not interfere with in vivo expression of tk when it is flanked by homologous 5' promoter sequences. The implication of these observations for retrovirus oncogenesis and animal cell genetics is discussed. (Author abstract) (40 Refs)

71. BK PAPOVAVIRUS INFECTIONS IN RENAL TRANSPLANT RECIPIENTS: CONTRIBUTION OF DONOR KIDNEYS.

Andrews C, Shah KV, Rubin R, Hirsch M
(c/o Shah) Dept. Pathobiology, The Johns Hopkins Univ. Sch. Hygiene and Public Health, 615 North Wolfe St., Baltimore, MD, 21205
J Infect Dis; 145(2):276 1982

Results with 230 subjects tested before and 6 months after receiving renal transplant for titer of HAI or neutralizing antibodies to BK papovavirus are reported. The seronegative recipient/seropositive donor combination had 3.5x the infection rate of the seronegative/seronegative combination. The seropositive recipient/seronegative donor and seropositive/seropositive combinations were at equal risk. A seropositive donor was shown to be more dangerous to a seronegative recipient than to a seropositive one. (3 Refs)

72. EFFECTS OF STEROIDS ON VIRAL INFECTION OF HUMAN BREAST CANCER CELL LINES BY ADENOVIRUS 5.

Chouvet C, Chardonnet Y, Saez S
Centre Leon Berard, 28 rue Laennec, 69372 Lyon Cedex 2, France
J Steroid Biochem; 15:269-274 1981

Effects of steroid hormones, especially estradiol (ES), on the infection of MCF7, a human epithelial cell line established from a metastatic breast adenocarcinoma, by adenovirus 5 were studied. Cytopathy induced by viral infection occurred 24 hr earlier in the presence of 10(-8) M ES. Some differences in the synthesis of viral proteins were detectable at 32 hr, and at 40 hr 78% of cells grown in the presence of hormone were positive vs 44% in the control. Potentiation of viral production is significant after 29 hr of infection and is max from 37 to 48 hr. The quantity of complete infectious particles produced in the control without hormone was very small for low virus concentrations [10 plaque forming units (PFU)/ cell] and increased with the dose of virus used. In the presence of ES, the difference with control was max for low infectious doses. Cells infected in the presence of 10(-9) M ES did not produce more viral particles than controls, but the addition of 10(-8) M ES amplified the number of PFU produced/cell approx 10x. Hormone deletion after infection did not decrease the production of viral particles. Whether the hormone was deleted for 2 or 4 hr before infection, the increase in viral production occurred as if hormone were present. The hormonal effect is apparently steroid specific and requires the corresponding receptors. More viral DNA is synthesized in the presence of hormone and max effect occurs later in the presence of hormone. No evidence was obtained for the synthesis of a protein specific to the hormonal effect, but the kinetics of synthesis were different between the hormone treated and untreated cultures. (26 Refs)

73. CIS AND TRANS-DIAMMINEDICHLOROPLATINUM(II) BINDING PRODUCES DIFFERENT TERTIARY STRUCTURAL CHANGES ON SV40 DNA.

Scovell WM, Kroos LR
Dept. Chemistry, Bowling Green State Univ., Bowling Green, OH, 43403
Biochem Biophys Res Commun; 104(4):1597-1603 1982

Tertiary structural changes produced by cis-diamminedichloroplatinum(II) (cisDDP) and trans-diamminedichloroplatinum(II) (trans-DDP) binding on simian virus 40 (SV40) DNA was studied by gel electrophoresis, assuming that the mobility of the DNA is proportional to the absolute number of supercoils. The Pt bound to DNA was determined by atomic absorption spectrophotometry after unbound Pt was separated by drop dialysis. Cis-DDP binding unwinds the supercoiled DNA to the intact relaxed form in about 6 hr, which is about 4x faster than observed for trans-DDP. When the SV40 DNA is incubated with the isomers for up to 30 min, cis-DDP reduces the mobility of supercoiled DNA at very short reaction times, an effect produced with trans-DDP only after an interval 50x longer. Results suggest that the cis-isomer exhibits a mode of binding different than that of the trans-isomer, a mode which is responsible for the more effective unwinding of the DNA. (29 Refs)

74. THE HUMAN INTERFERON SYSTEM.

Berman B, Frankfort HM
Dept. Dermatology, Mount Sinai Sch. Medicine, 1 Gustave L. Levy Place, New York, NY, 10029
Int J Dermatol; 21(1):12-18 1982

Clinical uses of interferon (IF) in dermatology are reviewed. IF inhibits both DNA- and RNA-containing viruses, as well as nonviral intracellular organisms. The immunomodulatory effects of IF on phagocytosis, histamine, prostaglandin E, antibody dependent cell-mediated cytotoxicity, natural killer lymphocytes, and mitogen activation of lymphocytes are discussed. Clinical studies of IF in cases of herpes zoster, varicella, herpes simplex, vaccinia, congenital rubella, condylomata acuminata and malignant melanoma are discussed. (85 Refs)

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VIRUS ISOLATION/ STRUCTURE/ CHARACTERIZATION/ IDENTIFICATION

1. THE STRUCTURE AND FUNCTION OF A MEMBRANE GLYCOPROTEIN ENCODED IN THE ADENOVIRUS GENOME.

Persson H, Philipson L
Dept. Microbiology, Biomedical Center, Box 581, S-751 23 Uppsala, Sweden

Protides Biol Fluid Proc Colloq; 29:97-100 1982

A glycoprotein encoded in the adenovirus genome interacts with the heavy chain of transplantation antigens. Sequence analysis of the E3/16,000 mol wt precursor protein obtained from adenovirus messenger RNA revealed a signal sequence in the N-terminal end and a hydrophobic sequence in the C-terminal end. Two sites are available for addition of carbohydrate to

HE 20.3173/2:

the side chains. It is now possible to identify the region which specifically interacts with HLA in human cells. (5 Refs)

2. STRUCTURAL DIFFERENCES BETWEEN HEXONS FROM ADENOVIRUS TYPES 2 AND 5: CORRELATION WITH DIFFERENCES IN SIZE AND IMMUNOLOGICAL PROPERTIES.

Von Bahr-Lindstrom H, Jornvall H, Althis S, Philipson L
Dept. Chemistry I, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden
Virology; 118(2):353-362 1982

The primary structure of the hexon capsid protein from adenovirus Type 5 (Ad5) has been investigated. The carboxymethylated polypeptide was cleaved with CNBr. The resulting fragments were purified and submitted to N-terminal sequence analysis. All large CNBr fragments were recovered and identified. Comparisons with the corresponding Ad2 peptides showed that the two hexon proteins have great similarities. Two regions with sequence differences were, however, identified, containing several amino acid substitutions. In addition, the Ad5 protein contained two major deletions. The smaller size of the Ad5 hexon can therefore be explained by internal deletions rather than by differences in the terminal ends. In accordance, the latter were largely unaltered between the two proteins. The regions that differ contain the trypsin sensitive sites of the native Ad2 hexon, explaining the insensitivity to trypsin of the native Ad5 protein. These sites may contain the type-specific immunological determinants of hexon. (Author abstract) (19 Refs)

3. DIFFERENT HISTONE FAMILIES IN INTRACELLULAR SV40 NUCLEOPROTEIN COMPLEXES WITH RESPECT TO THE ACETYLATION TURNOVER.

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Virology; 118(2):389-400 1982

The turnover of normal and butyrate-induced acetylation in the histones complexed with intracellular SV40 DNA was analyzed in infected CV-1 cells. The analysis concerned the two principal structures containing the intracellular SV40 chromatin: the 75 S complexes and the provirions. It was found that, while the normal acetylation in the 75 S chromatin turns over a lower rate than that of the host-cell chromatin, the hyperacetylation induced by butyrate turns over a rate very close to that of cell chromatin. This distinguishes two families of 75 S histones, presumably corresponding to nucleosomes with different accessibility to histone-modifying enzymes. A third type of turnover is shown by the provirion histones, in which the normal acetylation occurs at a low rate but is highly conserved. The response to butyrate in these structures is either absent or similar to that of the 75 S histones. In the chromatin of the late SV40 mutant tsB11, histone hyperacetylation does not occur when infection proceeds at the restrictive temperature for the coat-protein assembly. The hyperacetylation of histones which characterizes the morphogenesis of SV40 appears to be an assembly-dependent process, starting in the 75 S chromatin and building up mainly in the provirions. (Author abstract) (38 Refs)

4. NEW WAY TO ISOLATE SIMIAN VIRUS 40 NUCLEOPROTEIN COMPLEXES FROM INFECTED CELLS: USE OF A THIOL-SPECIFIC REAGENT.

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J Virol; 42(1):292-296 1982

A new method for the isolation of simian virus 40 nucleoprotein complexes from nuclei of lytically infected cells is described. The method is based on the addition of a thiol-specific reagent, 5,5'-dithiobis(2-nitrobenzoic acid), to lysis and extraction buffers. By inhibiting an uncoating activity during simian virus 40 extraction, 5,5'-dithiobis(2-nitrobenzoic acid) allows the use of efficient extraction buffers, such as ones containing Triton X-100 and EDTA, for the isolation of native simian virus 40 minichromosomes and virion-type structures. Use of the method is illustrated by following encapsidation of simian virus 40 minichromosomes in a pulse-chase experiment. Since 5,5'-dithiobis(2-nitrobenzoic acid) is an inhibitor of many different enzymes, the 5,5'-dithiobis(2-nitrobenzoic acid) extraction technique may be useful for the isolation of not only papovaviruses but also other viruses and possibly cellular chromatin. (Author abstract) (16 Refs)

5. USE OF RECOMBINANT PLASMIDS TO INVESTIGATE THE STRUCTURE OF THE HUMAN CYTOMEGALOVIRUS GENOME.

Oram JD, Downing RG, Akrigg A, Dollery AA, Duggleby CJ, Wilkinson GW, Greenaway PJ
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J Gen Virol; 59(part1):111-129 1982

Human cytomegalovirus (HCMV) DNA was digested with restriction endonucleases and the fragments characterized with respect to mol wt and relative mole proportions. The terminal fragments were identified by digesting HCMV DNA with exonucleases before restriction endonuclease treatment and subsequent gel analysis. The HindIII fragments of HCMV DNA were cloned in Escherichia coli and recombinant plasmids were characterized by digestion with restriction endonucleases and by molecular hybridization with HindIII, BglII and XbaI fragments of the virus genome. Data from these experiments were used to construct physical maps of HCMV DNA for the HindIII, BglII and XbaI restriction endonucleases. The terminal regions of the genome and the region containing fragment HindIII M were shown to be heterogenous. (Author abstract) (20 Refs)

6. ELECTRON MICROSCOPE STUDY OF EQUINE HERPESVIRUS TYPE 1 DNA.

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J Virol; 42(1):297-300 1982

Electron microscopic studies of equine herpesvirus DNA revealed that single strands that were allowed to reanneal formed single-stranded loops with double-stranded stems only at one end of the molecule. These observations support restriction enzyme analyses which indicate that the 92-megadalton DNA molecule exists as a long region of unique sequences covalently linked to a short region. The short region is comprised of an internal unique sequence, which forms the loop during reannealing of single strands, and two terminal inverted repeat sequences that bracket the unique sequence and form the double-

stranded stem structure observed upon reannealing of single strands. Measurements of the unique sequence and terminal inverted repeat subgenomic sequences indicate a size of 6.4 megadaltons for each and thus fix the size of the short region at approx 19.2 megadaltons. (Author abstract) (15 Refs)

VIRUS ASSOCIATED CELL TRANSFORMATION AND HOST ALTERATION

7. NUCLEOTIDE SEQUENCE ANALYSIS OF THE LINKED LEFT AND RIGHT HAND TERMINAL REGIONS OF ADENOVIRUS TYPE 5 DNA PRESENT IN THE TRANSFORMED RAT CELL LINE 5RK20.

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Nucleic Acids Res; 10(7):2189-2198 1982

A peculiar phenomenon is observed in several adenovirus Type 2 or 5 (Ad2 or Ad5) transformed cell lines: the right hand and left hand terminal regions of the viral genome present in the viral DNA insertions of these cell lines are found to be linked together. A large part of the viral DNA insertion present in the Ad5 transformed rat cell line 5RK20 has been cloned in the lambda vector Charon21A, including the segment containing the linked terminal regions. Sequence analysis of the linkage region showed a perfect homology with the Ad5 DNA sequence and a direct linkage of base pair (bp) 63 of the left hand end of the viral genome to bp 108 of the right hand end. No cellular or rearranged viral sequences were present. Our findings suggest that the joining of the terminal viral regions preceded the integration of the viral sequences into the cellular genome. (Author abstract) (21 Refs)

8. IDENTIFICATION AND PURIFICATION OF A PROTEIN ENCODED BY THE HUMAN ADENOVIRUS TYPE 2 TRANSFORMING REGION.

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J Virol; 42(1):30-41 1982

The human adenovirus Type 2 (Ad2) transforming genes are located in early regions E1a (map position 1.3 to 4.5) and E1b (map position 4.6 to 11.2). We have identified and purified to near homogeneity a major 20,000-mol-wt (20K) protein and have shown that it is coded by E1b. Using an Ad2-transformed cell antiserum which contained antibody to E1b-coded proteins, we immunoprecipitated 53K and 19K proteins from the nucleoplasm and 53K, 19K, and 20K proteins from the cytoplasmic S-100 fraction of Ad2 productively infected and Ad2-transformed cells. The 19K protein was present in both the nucleoplasm and the cytoplasm, where the 20K protein was found only in the cytoplasm. The 53K and 19K proteins are known Ad2 E1b-coded proteins. The 20K protein was purified to near homogeneity in 20 to 50% yields by sequential DEAE-Sephacel chromatography and reverse-phase high-performance liquid chromatography. Purified 20K protein shares most of its methionine-labeled tryptic peptides with E1b-53K, as shown by reverse-phase high-performance liquid chromatography, and therefore is closely related to the 53K protein. The 19K protein does not appear to share tryptic peptides with either 20K or 53K protein. To provide more direct evidence that 20K protein is virus-coded we translated E1b-specific mRNA in vitro. Both immunoprecipitation analysis and high-performance liquid chromatography purification of the translated product identified a 20K protein that has the same tryptic peptides as the 20K protein isolated from infected and from transformed cells. These findings suggest that the Ad2 20K protein is a primary translation product of an Ad2 E1b mRNA. (Author abstract) (39 Refs)

9. INTEGRATED ADENOVIRUS TYPE 12 DNA IN THE TRANSFORMED HAMSTER CELL LINE T637: SEQUENCE ARRANGEMENTS AT THE TERMINI OF VIRAL DNA AND MODE OF AMPLIFICATION.

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J Virol; 42(1):317-321 1982

Approx 20 to 22 copies of adenovirus Type 12 (Ad12) DNA per cell were integrated into the genome of the cell line T637. Only a few of these copies seemed to remain intact and colinear with virion DNA. All other persisting viral genomes exhibited deletions or inversions or both in the right-hand part of Ad12 DNA. Spontaneously arising morphological revertants of T637 cells had lost viral DNA. In most of the revertant cell lines only the intact or a part of the intact viral genome was preserved; other revertant cell lines had lost all viral DNA. In three other Ad12-transformed hamster cell lines, HA12/7, A2497-3, and CLAC3, major rearrangements at the right end of the integrated Ad12 DNA were not found. These studies were performed to investigate the phenomena of amplification, rearrangements, and deletions of Ad12 DNA in hamster cells. (Author abstract) (16 Refs)

10. UTILIZATION OF GAMMA-32P-GTP BY ECTOPROTEIN KINASE OF 3T3 AND SV40 3T3 CELLS.

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Cell Biol Int Rep; 6(4):385-392 1982

The utilization of GTP by the membrane ectoprotein kinase of 3T3 and SV40-transformed 3T3 cells (SV40 3T3) was investigated. Gamma-32P-GTP was utilized by 3T3 about 77% of that seen with ATP. The reaction was cyclic AMP and cyclic GMP-independent and required Mg ions for activity. A comparison of the phosphorylation of quiescent 3T3, serum-stimulated 3T3, and SV40 3T3 cells showed the same relative extent of phosphorylation by ATP and GTP: SV40 3T3 greater than stimulated 3T3 greater than quiescent 3T3. In each case, the amount of incorporation with GTP was less than that with ATP. Proteins from cells phosphorylated with labeled GTP or ATP were separated by polyacrylamide gel electrophoresis. Radioautography showed generally similar labeling patterns with the two nucleotides. However, quiescent 3T3 cells exhibited a greater than 165,000 dalton protein that was labeled more extensively with ATP than with GTP. This band might represent more than one protein. The equivalent band in serum-stimulated cells was labeled to the same extent with ATP or GTP, although not as pronounced as in the quiescent cells. The rest of the banding patterns were similar to those of the quiescent cells. Thus, the 3T3 and SV40 3T3 cell ectokinase is a membrane-related to cyclic AMP-independent enzyme which can utilize GTP as well as ATP to phosphorylate similar substrates. (19 Refs)

11. THE IDENTIFICATION OF A SERUM VIABILITY FACTOR FOR SV3T3 CELLS AS BIOTIN AND ITS POSSIBLE RELATIONSHIP TO THE MAINTENANCE OF KREBS CYCLE ACTIVITY.

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Arch Biochem Biophys; 215(2):556-563 1982

A factor isolated from calf serum using Sephadex G-25 gel filtration enhanced the viability and growth of SV40-transformed mouse 3T3 fibroblasts (SV3T3) grown in low-serum medium. This factor (peak III) was identified as biotin, based on the following observations: (1) peak III exhibited biological effects similar to biotin, eg reduction in the number of dead SV3T3 cells in culture, reduction in amount of lactic acid released by these cells, and a synergism with iron in stimulating growth; (2) following passage of peak III through an avidin-Sepharose affinity column, no viability/growth activity for SV3T3 was detected in the eluted fractions, but the activity was completely recovered following passage of peak III through a column of Sepharose alone; (3) peak III extracts reacted with p-dimethylaminocinnamaldehyde, a reagent which complexes with the cyclic ureide moiety of biotin; and (4) peak III exhibited the same relative front as authentic biotin on thin layer chromatography. When individual Krebs cycle intermediates were assayed for their ability to stimulate SV3T3 proliferation in low-serum medium not supplemented with biotin, malate, citrate, isocitrate, and fumarate were growth promoting, while alpha-ketoglutarate, oxaloacetate, and succinate were ineffective. These results are consistent with the hypothesis that biotin exerts its viability/growth promoting ability via cofactor activity with pyruvate carboxylase, thereby helping to maintain the levels of Krebs cycle intermediates. (20 Refs)

12. TRANSPORT OF 6-DEOXY-D-GLUCOSE AND D-XYLOSE BY UNTRANSFORMED AND SV40-TRANSFORMED 3T3 CELLS.

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Microbiology Section, Univ. Connecticut, Storrs, CT, 06268
J Cell Physiol; 111(1):83-88 1982

Serum-stimulated and SV-40-transformed 3T3 cells were observed to have similar high rates of transport of 6-deoxy-D-glucose and D-Xylose as compared to quiescent 3T3 cells. No enhancement of transport specific for transformation was found. Discrepancies in data from different laboratories on the transport of analogs of D-glucose by untransformed vs transformed 3T3 cells might be explained on the basis of differences in the growth state of the untransformed cells used in the experiments. (32 Refs)

13. PROPERTIES OF T ANTIGEN-POSITIVE PHENOTYPIC REVERTANTS ISOLATED FROM SV40-TRANSFORMED RAT AND MOUSE CELLS.

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Microbiol Immunol; 26(1):31-40 1982

T antigen-positive revertants isolated from SV40-transformed rat and mouse cells, which integrate six and two genome equivalents of viral DNA per cell respectively, were tested for tumorigenicity in nude mice and the relationship between tumorigenicity and karyological and growth properties of the cells was investigated. Three phenotypic revertants, R14, R15, R16, were isolated from W-3Y-23 cells. In soft agar medium, W-3Y-23 cells formed large colonies, R16 cells formed slightly smaller colonies, and neither R14 nor R15 formed large colonies. Revertant R14 showed a marked increase in the number of chromosomes to a median value of 130 and no subtetraploid cells were observed in their population. R15 and R16 showed a subtetraploid distribution similar to the parent cell line. W-3Y-23 cells were strongly tumorigenic and all the mice that received either high or low dose developed tumors within a wk. No tumors developed within 11 wk when approx 10(7) 3Y1-B, R14, or R15 cells were injected. R16 cells induced tumors that developed very slowly and became visible 8 wk after inoculation. All the tumors developed at the site of injection. R14, R15, and R16 cell DNA as well as W-3Y-23 DNA were cleaved with EcoRI which cuts the SV40 DNA once, and the DNA fragments containing the viral DNA sequence were analyzed. W-3Y-23 DNA generated seven distinct bands which were identical to those previously detected. Similar patterns were observed with all the revertant DNA; however the second and fourth bands from top of the gel were absent in all revertant DNA. In addition, the smallest band was generated from R14 and R15 DNA. The results indicate that a part of the viral DNA sequence had been deleted in all the revertants, while they still retained at least one complete viral genome. (19 Refs)

14. CHANGES IN PROSTAGLANDIN LEVELS IN CULTURES OF SV40-TRANSFORMED MACROPHAGE CELL LINES IN RELATION TO THEIR PHENOTYPIC EXPRESSION.

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Microbiol Immunol; 26(1):59-66 1982

Prostaglandin (PRG) levels were determined in culture fluids of simian virus 40-transformed cells, line 28-12 and its subline, and the release of PRG by the cells when they are treated with lipopolysaccharide (LPS) and OK-432 (a streptococcal preparation from penicillin-attenuated Streptococcus pyogenes) was investigated. Low levels of PRG were detected in Dulbecco's modified Eagle's medium, and significantly higher levels (4,921 +-140 picog/ml) were detected in heat-inactivated fetal calf serum. 28-12 cells were cultivated for 16 days and the medium was changed every 4 days. A low level of PRG was detected in 4-day culture medium during the growing phase of the cells. When the proportion of phagocytic cells increased during the stationary phase, a decrease in PRG levels was observed. The levels of PRG remained lower than background levels and constant during the culture period when the 28-12 subline isolated by cultivation with cytosine arabinoside (28-12Ara) was cultivated without a change in the medium for 4 days. Non-phagocytic 28-12 cells and phagocytic 28-12Ara cells were cultivated for 24 hr in the presence and absence of LPS (1 ug/ml) and OK-432 (25 ug/ml). The release of PRG by 28-12Ara cells was markedly enhanced, whereas its release by 28-12 cells was not affected. The results indicate the correlation between the phenotypic change from the nonphagocytic to the phagocytic state and a decrease in PRG levels in culture fluids, and that phagocytic cells are responsive to PRG inducers, whereas nonphagocytic cells are not. (27 Refs)

15. NUCLEOSOME SEGREGATION AT A DEFINED MAMMALIAN CHROMOSOMAL SITE.

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Proc Natl Acad Sci USA; 79(6,part1):1810-1814 1982

When animal cells replicate chromatin under conditions precluding new histone biosynthesis, half of the daughter DNAs are devoid of nucleosomes and are sensitive to staphylococcal nuclease. DNA sequences resistant to nuclease are associated with preexisting nucleosomes, which redistribute to progeny DNA duplexes during replication. We labeled newly replicated DNA sequences in a simian virus 40 (SV40)-transformed Chinese hamster cell clone with 5-bromodeoxyuridine (BrdUrd) in the presence and absence of a protein biosynthesis inhibitor, emetine. We resolved single-stranded BrdUrd- and dT-DNA sequences protected from nuclease digestion by nucleosomes and determined from which strands of the integrated viral DNA parental template (dT) and newly replicated progeny (BrdUrd) sequences were derived. Because we knew that the cell clone studied contained all of its integrated SV40 DNA at a single chromosomal site, we were able to determine that preexisting nucleosomes segregated to only one of the two daughter duplexes containing the integrated viral sequence. Additionally, in the presence of emetine, the integrated viral origin of replication, ORIsv, appeared not to function as a chromosomal replication origin, perhaps reflecting the drug's effect on synthesis of SV40 large tumor antigen. (Author abstract) (20 Refs)

16. NONSELECTIVE EXPRESSION OF SIMIAN VIRUS 40 LARGE TUMOR ANTIGEN FRAGMENTS IN MOUSE CELLS.

Reddy VB, Tevethia SS, Tevethia MJ, Weissman SM
Southern Medical and Pharmaceutical Corporation, 3500 East Fletcher Ave., Tampa, FL, 33612
Proc Natl Acad Sci USA; 79(6,part1):2064-2067 1982

To understand the role of various functional domains of simian virus 40 early tumor antigens, we have cloned and introduced into mouse cells portions of early simian virus 40 DNA. Two types of truncated large tumor antigen (33 and 12.3 kilodaltons), as well as small tumor antigen, were identified by immunoprecipitation. Both truncated large tumor antigens have been found to be overproduced with respect to the small tumor antigen, although the 12.3-kilodalton truncated large tumor antigen was more stable than the 33-kilodalton one. Nonviral 53-kilodalton protein was not found associated with either truncated large tumor antigen in immunoprecipitations. (Author abstract) (34 Refs)

17. INHIBITION OF EPIDERMAL GROWTH FACTOR BINDING TO MOUSE CULTURED CELLS BY FIBROBLAST-DERIVED GROWTH FACTOR. EVIDENCE FOR AN INDIRECT MECHANISM.

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J Biol Chem; 257(7):3680-3686 1982

Fibroblast-derived growth factor (FDGF), a basic, heat- and acid-stable polypeptide partially purified from the serum-free conditioned medium of BHK cells transformed by simian virus 40, is a potent mitogen for Swiss 3T3 cells and causes a marked reduction in 125I-labeled epidermal growth factor (125I-EGF) binding to these cells. The activity which inhibits EGF binding co-elutes with the growth-stimulating activity after gel filtration, ion exchange chromatography, and sodium dodecyl sulfate polyacrylamide gel electrophoresis. Both cellular responses are elicited by the same range of FDGF concentration in several murine cell types. The inhibition of EGF binding is rapid and results from a decrease in the apparent affinity of cellular receptors for 125I-EGF. FDGF does not affect the rate of cell-mediated 125I-EGF degradation. Several lines of evidence suggest that FDGF does not bind directly to EGF receptors. First, the effect of FDGF is dependent on the temperature of the assay; furthermore, treatment of cells with EGF results in loss of EGF receptors while exposure to FDGF from up to 24 hr does not induce 'down-regulation' of EGF receptors. Further, in A431 cells which display a large number of specific EGF receptors, 125I-EGF binding is not sensitive to FDGF. Finally, the effect of FDGF on 125I-EGF binding is not observed with isolated plasma membranes. Taken together, these findings suggest that FDGF binds to sites which are separate from EGF receptors. The results show a novel mechanism whereby a growth-promoting factor produced by a tumor cell line can rapidly modulate the affinity of the cellular receptors from EGF in an indirect manner. (Author abstract) (46 Refs)

18. ENHANCED METASTASIS OF TUMOURS INDUCED BY A SV40 SMALL T DELETION MUTANT.

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Dept. Biological Chemistry, Univ. California, Los Angeles, CA, 90024
Nature; 296(5858):672-675 1982

The ability of wild type SV40 (wt830) and a small T deletion mutant (d1884) to induce tumors in hamsters following sc injection was investigated. d1884 induced tumors at a similar frequency to wt830; however, the av latent period with d1884-induced tumors was 12.5 mo compared to 6.5 mo for wild type SV40. Some of the animals injected with d1884 developed tumor foci at sites distant from the injection site, while no distant foci were found in the animals with wt830-induced tumors. To determine whether the sc tumors and distant tumor foci actually arose from d1884-transformed cells, virus was rescued from cultured tumor cells by co-cultivation with permissive monkey cells (CV-1). CV-1 cells were then infected with the rescued virus and the viral DNA extracted from the cells. The HindII-cleaved or uncleaved viral DNA was electrophoresed on agarose gel. Viral DNA rescued from d1884-induced tumor cells co-electrophoresed with d1884 viral DNA, and viral DNA from wt830-induced tumors co-electrophoresed with wt830 DNA. To distinguish whether the distant tumor foci arose by metastases from a primary tumor or by independent virus-mediated transforming events, the viral genome integration patterns from cells of primary tumors and metastases were analyzed by hybridization of d1884 DNA restriction endonuclease fragments with an SV40 probe. The results showed that some distant tumor foci appear to be derived by metastasis from the primary sc tumor while the DNA integration patterns of other foci suggest that their development is unrelated to metastasis and may have arisen from an independent transforming event. (21 Refs)

19. CHANGES IN PHOSPHOLIPID POLAR HEAD GROUP TURNOVER IN SV40-TRANSFORMED HAMSTER FIBROBLASTS.

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FEBS Lett; 139(2):217-220 1982

The effect of viral transformation on turnover of phospholipid polar head groups was studied in vitro in hamster fibroblasts. Cell cultures of SVEHSVi hamster fibroblasts transformed by simian virus 40 (SV40) and cultures of nontransformed EHB hamster fibroblasts were labeled with 0.1 uCi/ml [U-14C]inositol, 0.5 uCi/ml [methyl-14C]choline, or 0.5 or 5 uCi/ml [2-14C]ethanolamine. Phospholipids from cell suspensions were separated by chromatography using chloroform/methanol solvent. Transformed cells incorporated inositol into phosphatidylinositol (PI) approx 2x as rapidly as nontransformed cells. Incorporation of ethanolamine into phosphatidylcholine (PC) and phosphatidylethanolamine (PE) was increased by a similar amount in transformed cells. Incorporation of choline into PC and sphingomyelin (SM) was decreased in transformed cells compared with nontransformed cells. Labeling of SM was delayed compared with labeling of PC. Only 1% of labeled ethanolamine was recovered in PC, indicating that not all PE was transmethylated into PC. Results indicate that transformed cells have a more rapid turnover of phospholipid polar head groups than do nontransformed cells. The significance of the turnover is uncertain, but may be related to cellular adhesive properties. (26 Refs)

20. EFFECTS OF PYRUVATE ON THE GROWTH OF NORMAL AND TRANSFORMED HAMSTER EMBRYO FIBROBLASTS.

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J Cell Physiol; 110(3):329-335 1982

The response of NIL1C1 hamster cells and their polyomavirus transformed counterpart, NILpy, to the presence of pyruvate in the growth medium was examined. NIL cells plated at low inoculum density (325 cells/square cm) responded to pyruvate by decreasing their population doubling time throughout a 6-day period of growth. The population doubling time decreased from about 23 hr in the absence of pyruvate to 11 hr in the presence of pyruvate. In contrast to NIL cells growing on medium containing glucose at 1 mg/ml, NIL cells at low inoculum density and 100 ug/ml glucose demonstrated only a small stimulation of growth when pyruvate was added. NIL cells also decreased glucose and glutamine utilization and increased lactate production in the presence of pyruvate. NILpy cells plated into medium containing 1 mg/ml glucose failed to respond to exogenously supplied pyruvate at any inoculum density. Lowering initial glucose concentration to 100 ug/ml resulted in NILpy cells responding to added pyruvate, but without inducing a significant change in population doubling time during logarithmic growth. NILpy cells in glucose medium also used constant amounts of glucose, and lactate production and glutamine and pyruvate utilization were also unaffected in inoculum density or the presence of pyruvate. (25 Refs)

21. DETECTION OF HERPES SIMPLEX VIRUS TYPE 2 GLYCOPROTEINS EXPRESSED IN VIRUS-TRANSFORMED RAT CELLS.

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J Virol; 42(1):275-282 1982

Rat embryo fibroblasts transformed by herpes simplex virus Type 2 (HSV-2) were assayed for the expression of certain virus-specific glycoproteins on the surface membranes. Monospecific antisera to HSV-2-specific glycoproteins, designated gAgB, gC, and gX, were used in membrane immunofluorescence studies with HSV-2-transformed cell lines tREF-G-1, tREF-G-2, and a tumor-derived rat fibrosarcoma cell line produced in syngeneic rats inoculated with tREF-G-1 cells. Analysis of the three HSV-2-transformed cell lines showed that antisera to the gAgB and gX glycoproteins were reactive with these cells. In contrast, no significant reactivity was observed when anti-gC serum was reacted with the HSV-2-transformed cell lines. All three antiglycoprotein sera reacted positively with rat cells productively infected with HSV-2. Additionally, the HSV-2-transformed and tumor-derived cell lines showed positive internal immunofluorescence after reaction with antiserum to an early, nonstructural viral protein designated VP143 (mol wt, 143,000). Infectivity of HSV-2 in standard plaque assays was neutralized by hyperimmune rat antisera to tREF-G-2 or rat fibrosarcoma cells and to HSV-2 virions and by sera from rats bearing the fibrosarcoma. Adsorption of rat anti-HSV-2 virions serum with tREF-G-2 or rat fibrosarcoma cells reduced neutralizing activity to 10 and 12%, respectively, compared with 90% neutralization by antiserum adsorbed with nontransformed rat embryo fibroblast cells and 100% neutralization with unadsorbed antiserum. In summary, HSV-2-transformed rat cells retained and expressed genetic information necessary for the production of HSV-2 glycoproteins and a nonstructural protein after high passage in tissue culture or in the syngeneic host. (Author abstract) (28 Refs)

22. ACTIVATION OF AN ENDOGENOUS MOUSE TYPE C VIRUS BY UV-IRRADIATED MURINE CYTOMEGALOVIRUS.

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Int J Cancer; 29(4):459-464 1982

Infection of Kirsten sarcoma virus-transformed non-producer BALB-3T3 mouse cells with UV-irradiated mouse cytomegalovirus (MCMV) resulted in activation of a xenotropic Type C virus detected by infectious center formation in permissive rat or mink cells. The levels of Type C virus activated by MCMV were related to the UV dose applied. Unter optimal conditions the frequencies of activation varied from 3.0 to 8.0 x 10(-4). The capacity of MCMV to activate Type C virus was abolished by heat inactivation and by neutralization with specific antiserum against MCMV. Virus induction decreased under conditions of cell exposure to hydroxyurea or actinomycin D, inhibitors of DNA and RNA synthesis, respectively, with actinomycin D having a greater inhibitory effect. This suggests that both DNA and RNA synthesis are required for UV-MCMV induction of murine xenotropic virus. (Author abstract) (33 Refs)

23. CELLULAR AND EPSTEIN-BARR VIRUS SPECIFIC DNA POLYMERASES IN VIRUS-PRODUCING BURKITT'S LYMPHOMA CELL LINES.

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Pharmacology Dept., Yale Univ. Sch. Medicine, New Haven, CT, 06510
Nucleic Acids Res; 10(7):2453-2465 1982

We have determined the levels of cellular DNA polymerases and Epstein-Barr virus specific DNA polymerase in three Burkitt's lymphoma cell lines producing varying amounts of EBV, one of which was induced by 12-O-tetradecanoylphorbol-13-acetate (TPA). There was a proportional increase in the level of EBV-DNA polymerase with an increase in the percent of virus-producing cells. However, there was a reciprocal relationship between the levels of EBV-DNA polymerase and DNA polymerase alpha ie, in cell line containing the highest level of EBV-DNA polymerase, activity of DNA polymerase alpha, but not of DNA polymerase beta, was reduced to an insignificantly low level. TPA does not have any direct effect on activities of either EBV-DNA polymerase or DNA polymerase alpha. EBV-DNA polymerases isolated from cells grown with or without TPA are indistinguishable in their properties such as elution position on phosphocellulose column, molecular weight, mono and divalent cation requirements, pH optimum, and other requirements for optimum activity. Addition of crude extracts of cells grown in presence of TPA to the purified DNA polymerase alpha did not inhibit its activity indicating that the observed loss was not due to any specific inhibitor present in TPA treated cells. Raji, a non-producer cell line, did not contain EBV-DNA polymerase. There was no induction of EBV-DNA polymerase when Raji cells were grown in presence of TPA. The phenomenon of reduction in the levels of DNA polymerase alpha in cells induced to produce EBV may represent a mechanism by which the host DNA replication is shut off following virus infection. (Author abstract) (36 Refs)

24. ENHANCED TUMOR GROWTH IN VIVO BY A FACTOR IN HUMAN PLATELETS AND RAT LIVER.

Witkoski F, Kepner N, Leitzel K, Rogers C, Jefferson LS, Lipton A (c/o Lipton) Div. Oncology, M. S. Hershey Medical Center, Pennsylvania State Univ., Hershey, PA, 17033
Cancer Res; 42(6):2350-2352 1982

Liver cell supernatant and platelet extract can promote the in vivo growth of herpes simplex virus type 2-transformed hamster embryo fibroblast cells. Delineation of necessary growth factors for tumor cells in vivo may open up new strategies to inhibit malignant cell growth. (Author abstract) (15 Refs)

VIRUS REPLICATION AND EXPRESSION

25. FUNCTION OF ADENOVIRUS TERMINAL PROTEIN IN THE INITIATION OF DNA REPLICATION.

Tamanoi F, Stillman BW
Cold Spring Harbor Lab., P. O. Box 100, Cold Spring Harbor, NY, 11724
Proc Natl Acad Sci USA; 79(7):2221-2225 1982

An early event in the initiation of adenovirus DNA replication is the formation of a covalent complex between the 87,000-dalton adenovirus terminal protein precursor and 5'-dCMP (pTP-dCMP complex). Nuclear extracts prepared from adenovirus-infected HeLa cells catalyzed complex formation in the presence of ATP, Mg++, and adenovirus DNA-protein complex but were not active when Pronase-treated DNA was used as template. The activity has been partially purified by chromatography on denatured DNA-cellulose and used to examine whether the 55,000-dalton terminal protein on adenovirus DNA is required for pTP-dCMP complex formation. Results obtained with either DNA-protein complex or Pronase-treated DNA were identical to those obtained using crude nuclear extracts. However, after treatment with piperidine to remove residual peptides, Pronase-treated DNA supported complex formation with the partially purified activity but not with crude extracts. In addition, when a plasmid containing an origin of adenovirus DNA replication was used as template, the pTP-dCMP complex was formed provided the plasmid was linearized in such a way that the origin was located at the end of the molecule. Neither linearized plasmid DNA with an internal origin nor supercoiled plasmid DNA supported complex formation. Furthermore, after heat denaturation, the linear plasmid DNA still supported complex formation, again provided that the origin was located at the end of the molecule. The partially purified protein fraction supported a limited amount of DNA chain elongation, which permitted exact positioning of the initiation site. These results suggest that enzymes responsible for complex formation recognize a DNA sequence at the origin and that the terminal protein on the template DNA plays a subordinate role. (Author abstract) (22 Refs)

26. PRESENCE OF THE PRE-MRNA FOR THE 72K DNA BINDING PROTEIN IN HNRNP FROM EARLY ADENOVIRUS-2 INFECTED HELA CELLS.

Kucherer C, Marty L, Blanchard J
Laboratoire de Biologie Moleculaire, Universite des Sciences et Techniques du Languedoc, Place E. Bataillon, 34060 Montpellier Cedex, France
Biochem Biophys Res Commun; 105(2):603-609 1982

An investigation was made on the RNa transcribed from adenovirus-2 (Ad-2) early region E2, which codes for the 72,000 dalton DNA binding protein (72K DBP). Tritium-labeled ribonucleoprotein particles (RNP) prepared from Ad-2 infected HeLa cells were deproteinized and the labeled RNA was hybridized to the EcoRI B DNA restriction fragment bound to a nitrocellulose filter. This DNA fragment carries the 3' half of the 72K DBP gene. EcoRI B specific RNA from nuclear RNP (hnRNP) was found in structures sedimenting in a sucrose gradient between 70 and 200S, while EcoRI B specific RNA from cytoplasmic RNA was found in a narrow distribution around 50S. Polyacrylamide gel electrophoresis of the RNA from the early Ad-2 transcripts showed a single 18S species in the cytoplasmic RNP in contrast to nuclear RNP, which showed a heterodisperse distribution of RNa in the 28S to 10S range. Previous studies indicate three size classes of nuclear RNA transcribed from the early region E2: a 28S pre-mRNA which is the first polyadenylated species likely to occur by cleavage of the primary transcript and which contains two intervening sequences, a 23S intermediate RNA and a 20S mature mRNA. These data indicate that, except for the species smaller than 18S which are degradation products, the newly synthesized intact transcripts of the gene coding for Ad-2 72K DBP are present in large hnRNP. Northern blot hybridization of RNA from early Ad-2 transcripts to EcoRI B DNA revealed that the EcoRI B probe hybridized to two components which correspond to the unspliced 28S pre-mRNA and to the mature 18S mRNA; the 23S transitory species was not observed. This demonstrates, in addition to the unspliced 28S pre-mRNA, the existence of mature mRNA molecules in nu-

clear RNP which differ in sedimentation coefficient from cytoplasmic mRNP. (30 Refs)

27. TRANSCRIPTION OF ADENOVIRUS CORES IN VITRO: MAJOR RNA PRODUCTS DIFFER FROM THOSE MADE FROM A DNA TEMPLATE.

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Dept. Molecular Biology, Univ. California, Berkeley, CA, 94720
Proc Natl Acad Sci USA; 79(6,part1):1834-1838 1982

Intact cores of adenovirus type 5 were transcribed in vitro in extracts derived from uninfected HeLa cells, and the products were analyzed by hybridization to restricted viral DNA. Predominant transcripts from cores were distinctly different from transcripts generated from deproteinized DNA. The extracts contained RNA polymerase II and RNA polymerase III. Transcription from cores was completely inhibited by low levels of alpha-amanitin, and there was little or no hybridization of products to the region of the genome encoding the small polymerase III-dependent virus-associated RNA that was major transcript from the DNA template. The major transcripts from cores hybridized to the rightward-transcribed strand of the region between 75 and 89 map units, early region 3 of the adenovirus genome. There was several-fold more transcription of this region in cores than of the region downstream from the major late promoter, which predominates among polymerase II transcripts from DNA. (Author abstract) (38 Refs)

28. ALTERED CELL CYCLE PROGRESSION AND ABERRANT MITOSIS IN ADENOVIRUS-INFECTED RODENT CELLS.

Murray JD, Bellett AJ, Braithwaite AW, Waldron LK, Taylor IW
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J Cell Physiol; 111(1):89-96 1982

It is reported that human adenovirus type 5 (Ad5) caused alterations in the cell cycle of some growing rodent cells, and that the induction of chromosome damage was cell-cycle dependent and required the expression of one or more early viral genes. Temperature sensitive mutants of Ad5 were found incapable of viral DNA replication and hence gene expression at not permissible temperatures without affecting chromosome damage; this damage must thus result from expression of one or more of the early viral genes. Ad5-infected cells suffered mitotic abnormalities that affected cells suffered mitotic abnormalities that affected segregation of chromosomes and cytokinesis as well as structural aberrations in the chromosomes. Chromosome damage was higher in all Ad5-infected samples than in mock-infected controls. Cyclic variations in virus-induced damage occurred with peaks averaging 22 hr apart. The av total cell cycle time calculated for mock-infected cells was 26 hr and for Ad5-infected cells 20 hr. G2, M and S phases were approximately the same for the two populations. Differences in cell cycle time were therefore due to differences in the length of F1, which was approx 5 hr in control cells and 0.5 hr in Ad5-infected cells. (26 Refs)

29. ADENOVIRAL PROTEIN-PRIMED INITIATION OF DNA CHAINS IN VITRO.

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Dept. Molecular Genetics, Inst. Plant Virus Res., Tsukuba Science-City, Yatabe, Ibaraki 305, Japan
Proc Natl Acad Sci USA; 79(8,part1):2442-2446 1982

The initiation of DNA chains by the 80-kilodalton form of the adenovirus terminal protein has been studied. This protein, which can be covalently linked to dCMP, is isolated complexed to a 140-kilodalton protein possessing DNA polymerase activity. In the presence of adenovirus DNA-protein, the formation of the 80-kilodalton protein-dCMP complex requires the addition of ATP and nuclear extract from uninfected cells in addition to Mg++ and dCTP. When single-stranded DNA is used in place of the adenovirus DNA-protein, the formation of the 80-kilodalton protein-dCMP complex occurs in the absence of ATP and nuclear extract. In the presence of the four dNTPs, the complex yields DNA chains of various sizes between 100 and 300 nucleotides. The products formed with bacteriophage phiX174 single-stranded circular DNA as the template are site specific, predominantly derived from the sequences between nucleotides 2363 and 2977 and between nucleotides 3760 and 4206. These small DNA chains are blocked at their 5' ends with the 80-kilodalton protein but possess free 3'-OH ends that are susceptible to degradation by exonuclease III and can be elongated to replicative form II products with DNA polymerase I of Escherichia coli or eukaryotic DNA polymerase beta preparations. A protein priming model explaining the different requirements for initiation with adenovirus DNA-protein and with phiX174 DNA is presented. (Author abstract) (20 Refs)

30. POLYPEPTIDES ENCODED BY TRANSFORMING REGION E1B OF HUMAN ADENOVIRUS 2: IMMUNOPRECIPITATION FROM TRANSFORMED AND INFECTED CELLS AND CELL-FREE TRANSLATION OF E1B-SPECIFIC MRNA.

Matsuo T, Wold WS, Hashimoto S, Rankin A, Symington J, Green M
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Virology; 118(2):456-465 1982

We have identified polypeptides specific to region E1b (map position [mp] 4.6-11.2) of adenovirus 2 (Ad2) that are synthesized in six lines of Ad-transformed rat or human cells (F17, F4, T2C4, 8617, 5RK clone I, 293), and in Ad2 early infected KB cells. [35S]Methionine-labeled polypeptides were immunoprecipitated using antisera against F17 cells, an Ad2-transformed rat cell line that retains only E1. To determine whether they are viral coded, these polypeptides were compared by tryptic peptide mapping with polypeptides translated in vitro from Ela-specific mRNA (mp 1.3-4.5) and E1b-specific mRNA. Polypeptides of 19,000 daltons (19K), 20K, and 53K were immunoprecipitated from Ad2 early infected KB cells. The 19K, 20K, and 53K could be translated from E1b-specific mRNA and thus are coded by E1b. The 19K was precipitated from all transformed cell lines, the 20K was immunoprecipitated from F4, 8617, and T2C4 cells, and the 53K was immunoprecipitated from F4, 8617, T2C4, and 293 cells. These results suggest that the 19K, and perhaps the 20K and 53K, may be important in adenovirus-induced cell transformation. The 20K and 53K share methionine-containing tryptic peptides with each other, but not with the 19K. These results, together with the Ad2 E1b DNA sequence suggest that 19K is translated in a different reading frame from 53K and 20K. (Author abstract) (39 Refs)

31. ON THE DYNAMICS OF THE MICROFILAMENT SYSTEM IN HELA CELLS.

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Div. Biology, California Inst. Technology, Pasadena, CA, 91125
J Cell Biol; 93(1):122-128 1982

We measured the pools of unpolymerized and filamentous actin in homogenates of HeLa cells made in several different lysis buffers, as well as after treatment of cells with a variety of chemicals or trypsin, and after adenovirus (Type 2) infection. This was possible when a series of factors concerning the basic culture conditions were kept constant: eg, serum type used, serum batch, cell density, time after subcultivation of cells, and buffering substance in the medium. Homogenates from untreated cells usually contain 35-45% of the total actin in an unpolymerized form. With some batches of cells this number can be as high as 50%. In sparse cultures (3 x 10(4) cells/cm2), HeLa cells contain approx 10 pg actin/cell, while the corresponding number is only 5 pg in dense cultures (3 x 10(5) cells/cm2). Treatment of cells with cytochalasin B increases the pool of unpolymerized actin by approx 30-40%, while colchicine decreases the fraction of unpolymerized actin by 20%. The oxidant diamide increases the filamentous actin pool 25-50%. Glucose, sodium azide, dinitrophenol, serum starvation, or thymidine treatment does not affect the distribution between unpolymerized and filamentous actin to any significant extent. Trypsin and EDTA induced rounding up of cells but did not change the actin distribution. The distribution of actin between G- and F-forms was unchanged after adenovirus infection. These results show that significant changes in the actin pools can be induced in nucleated cells. However, several treatments which alter the morphology and motility of cells are not accompanied by an alteration in the G-/F-actin ratio. (Author abstract) (29 Refs)

32. INITIATION OF SV40 DNA REPLICATION IN VIVO: LOCATION AND STRUCTURE OF 5' ENDS OF DNA SYNTHESIZED IN THE ORI REGION.

Hay RT, DePamphilis ML
Medical Res. Council Virology Unit, Church St., Glasgow, G11 5JR, Scotland
Cell; 28(4):767-779 1982

Initiation sites for DNA synthesis were located at the resolution of single nucleotides in and about the genetically defined origin of replication (ori) in replicating SV40 DNA purified from virus-infected cells. About 50% of the DNA chains contained an oligoribonucleotide of six to nine residues covalently attached to their 5' ends. Although the RNA-DNA linkage varied, the putative RNA primer began predominantly with rA. The data reveal that initiation of DNA synthesis is promoted at a number of DNA sequences that are asymmetrically arranged with respect to ori: 5' ends of nascent DNA are located at several sites within ori, but only on the strand that also serves as the template for early mRNA, while 5' ends of nascent DNA with the opposite orientation are located only outside ori on its early gene side. This clear transition between discontinuous (initiation sites) and continuous (no initiation sites) DNA synthesis defines the origin of bidirectional replication at nucleotides 5210-5211 and demonstrates that discontinuous synthesis occurs predominantly on the retrograde arms of replication forks. Furthermore, it appears that the first nascent DNA chain is initiated within ori by the same mechanism used to initiate nascent DNA ('Okazaki fragments') throughout the genome. (Author abstract) (63 Refs)

33. AN SV40 DELETION MUTANT ACCUMULATES LATE TRANSCRIPTS IN A PARANUCLEAR EXTRACT.

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Virology; 119(1):1-11 1982

We have constructed a deletion in the SV40 late region which has lost the sequences between nucleotides 226 and 656. This deletion has lost most of utilized 5' ends, leaders, all of the intervening sequence and body acceptor site for late 19 S mRNA. We show that the transcripts made by this deletion are concentrated in a paranuclear extract relative to other subcellular fractions. Furthermore, the late transcripts of the helper virus used to grow this mutant are also concentrated in the paranuclear extract. This observation leads us to speculate that these mutant transcripts may poison the RNA transport process. (Author abstract) (33 Refs)

34. INTRACELLULAR TRANSPORT OF SV40 LARGE TUMOR ANTIGEN: A MUTATION WHICH ABOLISHES MIGRATION TO THE NUCLEUS DOES NOT PREVENT ASSOCIATION WITH THE CELL SURFACE.

Lanford RE, Butel JS
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Virology; 119(1):169-184 1982

The cT mutation of PARA, an SV40-adenovirus 7 hybrid virus, abolishes the transport of SV40 T-antigen (T-ag) to the nucleus and results in the accumulation of T-ag in the cytoplasm of infected and transformed cells. The effect of the cT mutation on the association of T-ag with the cell surface was examined in PARA(cT)-infected monkey cells. Surface-associated T-ag was detected by immunofluorescence, lactoperoxidase-catalyzed cell-surface iodination, and cellular fractionation. Surface-associated T-ag was more readily labeled by cell-surface iodination with PARA(cT)-infected cells than with SV40 and wild-type PARA [PARA(nT)]-infected cells. Control experiments eliminated the possibility that the enhanced detection of T-ag in PARA(cT)-infected cells was due to the labeling of intracellular T-ag by lactoperoxidase or to T-ag leaking from dead cells and adhering to the cell surface. The elevated level of surface-associated T-ag in PARA(cT)-infected cells was confirmed by metabolic labeling and cellular fractionation, indicating that the increased iodination of surface-associated T-ag was due to a greater number of T-ag polypeptides on the surface rather than a greater exposure of tyrosine residues. Pulse-chase experiments demonstrated that the elevated level of surface-associated T-ag in PARA(cT)-infected cells was not due to a prolonged stability of surface-associated T-ag. The cT mutation provides a useful tool for studies of mechanisms governing intracellular protein transport. (Author abstract) (47 Refs)

35. AN OLIGORIBONUCLEOTIDE POLYMERASE FROM SV40-INFECTED CELLS WITH PROPERTIES OF A PRIMASE.

Kaufmann G, Falk HH
Biochemistry Dept., Weizmann Inst. Science, Rehovot 76100, Israel
Nucleic Acids Res; 10(7):2309-2321 1982

A transient decaribonucleotide (iRNA) is covalently linked to nascent eukaryotic DNA chains at their 5' end. Searching for the putative iRNA polymerase (primase), we detected in extracts from SV40-infected cells a DNA-dependent incorpora-

tion of UMP residues from UTP into free and DNA linked deca- or similarly sized ribonucleotides. Denatured salmon sperm DNA served as the standard template in this reaction. SV40 FIII DNA was also an effective template, SV40 FII DNA was ineffective while FI yielded mainly free decaribonucleotides. The incorporation depended on the other rNTPs and was resistant to high concentrations of alpha-amanitin and rifamycin AF/013, drugs inhibitory to RNA polymerases I, II and III. The results implicate the decaribonucleotide polymerase in the priming of nascent DNA chains and suggest that the unique size of iRNA is encoded within its primase. (Author abstract) (27 Refs)

36. CONDITIONS FOR SLIDING OF NUCLEOSOMES ALONG DNA: SV 40 MINICHROMOSOMES.

Glotov BO, Rudin AV, Severin ES
Inst. Molecular Biology, USSR Acad. Sciences, Moscow 117984, USSR
Biochim Biophys Acta; 696(3):275-284 1982

Sliding of nucleosomes along DNA under nearly physiological conditions was studied using treatment of SV 40 minichromosomes with the single-cut restriction endonucleases EcoRI and BamHI. Each enzyme can convert no more than 20-25% of the circular DNA molecules of minichromosomes into the linear form irrespective of the presence of histone H1. This suggests absence of the nucleosome lateral migration (sliding) along DNA at least in the vicinity of the restriction endonuclease cleavage sites during several hr of incubation. The sites available for EcoRI and BamHI in minichromosomes seem to be located predominantly in the spacer DNA regions of nucleosomes. Introduction of only one double-strand (but not single-strand) break into the DNA of minichromosomes stripped of histone H1 is sufficient to induce redistribution of the nucleosome core particles due to their sliding along DNA. Thus, sliding of the nucleosome core particles can be induced under physiological conditions by rather low energy expenditures. (Author abstract) (30 Refs)

37. SIMIAN VIRUS 40-RABBIT BETA-GLOBIN RECOMBINANTS LACKING LATE MRNA SPLICE SITES EXPRESS CYTOPLASMIC RNAS WITH ALTERED STRUCTURES.

White RT, Berg P, Villarreal LP
Dept. Biochemistry, Univ. California, Berkeley, CA, 94720
J Virol; 42(1):262-274 1982

Deletions were introduced at exon-intron boundaries in the late region of a simian virus 40-beta-globin cDNA recombinant to study the role of splicing in the formation of simian virus 40 late cytoplasmic RNAs. The recombinant was used as a wild type because it allowed characterization of mutant RNAs expressed from defective genomes in the presence of comparable RNAs contributed by the coinfecting helper virus. Removal of a 17-base pair segment at map position 0.76, which included a portion of the leader sequence implicated in the splicing of the major 16S mRNA, prevented expression of 16S-type mRNA. The same mutant accumulated cytoplasmic 19S-type mRNA, but the assortment of the 5' ends of these mRNAs differed from the assortment in the wild-type counterparts. Another mutant that lacks nucleotide sequences implicated in the splicing of the major 16S mRNA and one of the principal 19S-type RNAs accumulated a 16S-type mRNA with a previously undetected leader splice, an assortment of 19S mRNAs with new or normally underrepresented splices, and even a species of unspliced cytoplasmic 19S mRNA. (Author abstract) (36 Refs)

38. VIABLE POLYOMA VIRUS VARIANT WITH TWO ORIGINS OF DNA REPLICATION.

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Virology; 119(1):12-21 1982

A viable evolutionary variant of polyoma virus, ev 1001, produced small clear plaques in assays on mouse 3T3 cells. The kinetics of virus formation during a single infection cycle showed that progeny virus was produced more rapidly than in cells infected by wild-type virus. However, an early cytopathic effect on the infected cells appeared to interrupt the process, since the yield of virus was low. Infection of semipermissive rat cells by ev 1001 virus resulted in unusually high transformation frequencies, particularly at low multiplicities of infection. The biological properties of ev 1001 can all be explained by the effect of a duplication of a 250-base pair segment of the polyoma genome in a region located between the 5'-ends of the early and late genes. The evolutionary variant has two origins of DNA replication, as judged by the high rate of DNA synthesis and strong interference with the formation of wild-type virus in coinfection experiments. The variant ev 1001 also induced mouse cells to synthesize cellular DNA more efficiently than wild-type virus. This effect might result from an increased expression of the viral early genes. (Author abstract) (38 Refs)

39. DETAILED STRUCTURAL ANALYSIS OF TWO SPLICED HSV-1 IMMEDIATE-EARLY MRNAS.

Rixon FJ, Clements JB
Inst. Virology, Univ. Glasgow, Glasgow G11 5JR, Scotland
Nucleic Acids Res; 10(7):2241-2256 1982

The structures of two HSV-1 immediate-early mRNAs have been determined by nuclease-digestion procedures using 5' and 3' end-labeled DNA probes. These mRNAs, which map across the junctions between the short unique (US) and short repeat (IRS and TRS) genome regions, have common 5' portions located in IRS and TRS. The 3' portions, which extend into opposite ends of US, are unique. The DNA sequence encoding the common 5' portions largely comprises a 247 base pair (bp) leader region and a single intron of variable size. The variation in intron length is due to different copy numbers of a 22 bp tandem reiteration. A small proportion of the mRNA population is unspliced, but otherwise is identical to the more abundant spliced species. (Author abstract) (38 Refs)

VIRAL GENETICS

40. TRANSFORMATION-DEFICIENT ADENOVIRUS MUTANT DEFECTIVE IN EXPRESSION OF REGION 1A BUT NOT REGION 1B.

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J Virol; 42(1):106-113 1982

An adenovirus Type 5 host range mutant (hr440) has been isolated which is defective in a splicing event required to generate the middle-sized mRNA from early region 1A. This defect has been ascribed to two adjacent nucleotide changes which lie five and six nucleotides from the 5' splice site for this mRNA. One of these changes introduces an amber codon into

the reading frame of the largest region 1A mRNA, resulting in the production of a truncated polypeptide. Like other region 1A mutants, hr440 is defective in the production of mRNA from early regions 2 and 3, but hr440 is unusual in that transcription from regions 1B and 4 is normal. Furthermore, although region 1B expression is unaffected, hr440 does not transform baby rat kidney cells. Therefore, expression of early region 1B is insufficient for transformation, eliminating the possibility that region 1A is required only to induce such expression. (Author abstract) (41 Refs)

41. ISOLATION AND PRELIMINARY CHARACTERIZATION OF A PHOSPHONOACETIC ACID-RESISTANT AND TEMPERATURE-SENSITIVE MUTANT OF HERPES SIMPLEX VIRUS TYPE I.

Daksis JI, Priemer MM, Chan VL
Dept. Microbiology and Parasitology, Univ. Toronto, Toronto, Ontario M5S 1A1, Canada
J Virol; 42(1):20-29 1982

A group of 43 phosphonoacetic acid (PAA)-resistant mutants of herpes simplex virus Type 1 was isolated after the mutagenesis of infected cells with nitrosoguanidine. One of these mutants, designated PAA1rts1, was found to be temperature sensitive (ts), that is, unable to replicate at 39.5 C, the nonpermissive temperature. Recombination analysis of PAA1rts1 indicated that the PAA1r mutation and the ts1 mutation are loosely linked and are located on two separate genes. PAA1rts1 showed a defect in viral DNA synthesis at 39.5 C, which presumably can be attributed to the production of a PAA-resistant and thermolabile DNA polymerase. PAA1rts1 was also defective in the shutoff of host DNA synthesis at the restrictive temperature. (Author abstract) (20 Refs)

IMMUNOLOGICAL STUDIES

42. GENERATION OF CYTOTOXIC LYMPHOCYTES BY SV40-INDUCED ANTIGENS.

Chang C, Chang R, Mora PT, Hu C
Dept. Medical Res., Veterans General Hosp., Taipei, Taiwan, Republic of China
J Immunol; 128(5):2160-2163 1982

In order to study the correlation of in vivo tumor transplantation immunity and in vitro immunologic assays, cell-mediated cytotoxicity against SV40-transformed cells was studied in AL/N strain mice by using 51Cr-release assay. Killing of SV40-transformed AL/N fibroblast cells was observed by spleen cells of AL/N mice immunized with syngeneic SV40-transformed cells. Immunization with the solubilized SV40 tumor-specific transplantation antigen (TSTA) that induced transplantation immunity in vivo did not elicit cytotoxic spleen cells in vitro. However, the spleen cells from mice immunized with solubilized TSTA and then sensitized in vitro with SV40-transformed cells became cytotoxic against SV40-transformed fibroblasts. Similarly, SV40 TSTA (T antigen) purified by immunoprecipitation was able to prime the lymphocytes in AL/N mice: the primed lymphocytes could differentiate into cytotoxic lymphocytes upon in vitro stimulation by SV40-transformed cells. These data indicate that SV40 TSTA (T antigen) plays a role in the induction of cytotoxic lymphocytes. (Author abstract) (30 Refs)

43. SPLIT T-CELL TOLERANCE IN HERPES SIMPLEX VIRUS-INFECTED MICE AND ITS IMPLICATION FOR ANTI-VIRAL IMMUNITY.

Nash AA, Ashford NP
Dept. Pathology, Univ. Cambridge, Tennis Court Road, Cambridge, England
Immunology; 45(4):761-767 1982

Mice simultaneously injected intravenously and subcutaneously with herpes simplex virus fail to adoptively transfer delayed hypersensitivity (DH) to syngeneic recipients. The transferred lymph node cells also failed to rapidly eliminate infectious herpes from the pinna, despite the presence of cytotoxic T cells in the transferred suspension. Both primary and secondary cytotoxic cell responses in the draining lymph node were unaffected by the inhibition of DH. The lymph nodes from DH tolerized mice also contain lymphocytes capable of undergoing a proliferative response in vitro to herpes antigens. In addition, a neutralizing antibody response with IgG antibodies against herpes are also present in DH tolerized mice. These data suggest a form of split T-cell tolerance in which only DH responses are directly compromised. The implication of these findings for the pathogenesis of herpes simplex virus is discussed. (Author abstract) (13 Refs)

44. MONOCLONAL ANTIBODIES DETECTING EPSTEIN-BARR VIRUS STRAIN AND GROUP SPECIFIC ANTIGENIC DETERMINANTS.

Herbst H, Georg-Fries B, Muller-Lantzsch N, Hausen HZ, Braun DG
Pharmaceuticals Res. Div., CIBA-GEIGY, Basel, Switzerland
Protides Biol Fluid Proc Colloq; 29:735-738 1982

The ability of monoclonal antibodies to detect common and group- and strain-specific antigenic sites on Epstein-Barr virus (EBV) proteins was examined. The EBV strain QIMR-WIL expressing four major envelope polypeptides of mol wt 80,000 (p80), 140,000 (p140), 240,000 (p240), and 340,000 (p340) was used for immunization. Ten of 144 culture supernatants exhibited clearly positive reactions, while 21 gave a weak immunofluorescence. One culture fluid precipitated polypeptide p80 exclusively, while others after cloning precipitated selectively p140, p340, or p240/p340 in combination. Cross-reactions with corresponding polypeptides of EBV strains P3HR-1 and B95-8 were studied. It was suggested that monoclonal antibodies can provide a serological basis for EBV typing. (9 Refs)

45. EPSTEIN-BARR VIRUS INCREASES THE PROLIFERATIVE RESPONSE AND THE GENERATION OF SUPPRESSOR AND CYTOTOXIC T-CELL FUNCTIONS IN AUTOLOGOUS MIXED LYMPHOCYTE REACTION.

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Scand J Immunol; 15(1):17-24 1982

Epstein-Barr virus (EBV) infection of stimulator cells significantly increased the proliferative response of T cells in the autologous mixed lymphocyte reaction (AMLR). The addition of a monoclonal anti-HLA-DR antibody to AMLR cultures in which either EBV-infected or non-infected non-T cells were used as stimulator cells strongly inhibited the proliferative response irrespective of the presence of EBV. It is concluded that EBV does not by itself activate the responding cells and that HLA-DR antigens are necessary to trigger T cells. Increased generation of suppressor T cells, determined in both alloantigen-induced DNA synthesis and pokeweed-mitogen-stim-

ulated immunoglobulin production, was found after an EBV infection of stimulator cells. Similarly, EBV-infected non-T cells significantly increased the generation of killer T cells, determined in three different types of target cells: phytohemagglutinin-stimulated mononuclear cells, EBV-transformed cells, and concanavalin-A-activated murine spleen cells. The increased T-cell responses after an EBV infection may reflect the attempts in vivo to control and hold in check the viral infection. (Author abstract) (33 Refs)

46. EARLY GROWTH OF EXPERIMENTAL TUMORS AND THE ONSET OF CONCOMITANT IMMUNITY.

Karakousis CP, Paolini NS, Sharma SD, Moore RH
Roswell Park Memorial Inst., 666 Elm St., Buffalo, NY, 14263
Eur J Cancer Clin Oncol; 18(2):211-217 1982

On repeated experiments, the rate of take of a given threshold inoculum of T241, B-16, SV40, L1210 or rat Wilms tumor in their syngeneic strains was not increased by additional tumor implanted concomitantly in other site(s), suggesting that early tumor growth is not dependent on an overwhelming by the tumor load of systemic host factors. There is no evidence in vivo of immune response in allogeneic or syngeneic tumors initially, following inoculation. Such evidence, in the form of tumor rejection or decreased rate of challenge take, does not appear at any definite interval from the time of inoculation, but its onset varies with the size of initial inoculum and coincides approx with the time of early palpability of the tumor nodule. The take of a threshold inoculum apparently depends on its interaction with local host factors. (Author abstract) (16 Refs)

OTHER RELATED STUDIES

47. INTERVENING EFFECT OF L-ASCORBIC ACID ON EPSTEIN-BARR VIRUS ACTIVATION IN HUMAN LYMPHOBLASTOID CELLS AND ITS COMPARISON WITH THE EFFECT OF RETINOIC ACID.

Morigaki T, Ito Y
Dept. Microbiology, Faculty Medicine, Kyoto Univ., Kyoto 606, Japan
Cancer Lett; 15(3):255-259 1982

Inhibitory effects of L-ascorbic acid (AA) on the induction of Epstein-Barr virus (EBV)-associated antigens in human lymphoblastoid P3HR-1 and Raji cells latently infected with EBV were studied. The effect of AA was compared with the inhibitory effect of retinoic acid (RA) in the same system; the intervening activity of AA was weaker but its cytotoxicity was remarkably lower than that of RA. The effect of AA in varying concentrations (0.1-1,000 uM) on the induction of EBV early antigen and viral capsid antigen in the producer P3HR-1 cell system by single use of 5-iododeoxyuridine, n-butyrate, or 12-O-tetradecanoyl-phorbol-13-acetate is illustrated. (10 Refs)

48. REACTIVATION OF UV AND GAMMA-INACTIVATED HERPES VIRUS IN BHK CELLS.

Glazier KG, Weber MC, Evans HH
Div. Radiation Biology, Dept. Radiology, Case Western Reserve Univ., Cleveland, OH, 44106
Mutat Res; 93(2):263-271 1982

The host-cell reactivation of irradiated Herpes simplex virus Type 1 (MacIntyre strain) (HSV1) baby hamster kidney (BHK) cells was compared to that of mammalian cells previously shown to be efficient in this process: normal human fibroblasts (HF) and UV-repair-deficient xeroderma pigmentosum (XP) cells. BHK cells had an intermediate ability to reactivate UV-damaged virus (HF greater than BHK greater than XP). BHK cells were more proficient at DNA repair than mouse L929 fibroblasts when UV dose as low, but were less capable than mouse cells at higher doses. HF and BHK were similar to each other in reactivation of gamma-irradiated HSV1 but XP cells were somewhat less efficient than the other two types. UV treatment of BHK cells 0-48 hr prior to infection with HSV1 enhanced the reactivation of the virus. The relationship between bacterial excision repair and reactivation of virus by mammalian cells is discussed. (35 Refs)

49. COMPARATIVE EFFICACY OF ANTIHERPES DRUGS IN DIFFERENT CELL LINES.

De Clercq E
Rega Inst. Medical Res., Univ. Leuven, B-3000 Leuven, Belgium
Antimicrob Agents Chemother; 21(4):661-663 1982

A selection of antiherpes compounds including bromovinyldeoxyuridine (BVDU), acyclovir (ACV), and fluoroiodoaracytosine (FIAC) were compared for their inhibitory effects on herpes simplex virus type 1 (strain KOS) replication in a wide variety of human, simian, feline, and murine cell lines. The 50% inhibitory doses of the compounds varied considerably from one cell line to another, ie, from 0.01 to 2 ug/ml for ACV and from 0.004 to 0.2 ug/ml for BVDU and FIAC. However, the relative order of antiviral potency remained constant, thus (in order of decreasing potency) BVDU greater than FIAC greater than ACV, for all cell lines studied except for the murine embryo cells, in which the order of decreasing potency was BVDU greater than ACV greater than FIAC, and for the feline lung cells, in which the order of decreasing potency was FIAC greater than ACV greater than BVDU. (Author abstract) (10 Refs)

50. ALPHA INTERFERON AND ACYCLOVIR TREATMENT OF HERPES SIMPLEX VIRUS IN LYMPHOID CELL CULTURES.

Hammer SM, Kaplan JC, Lowe BR, Hirsch MS
Infectious Disease Unit, Dept. Medicine, Massachusetts General Hosp., Boston, MA, 02114
Antimicrob Agents Chemother; 21(4):634-640 1982

The T-lymphoblastoid cell line CEM, persistently infected with herpes simplex virus type 1, has been used to examine the antiviral efficacy of human alpha interferon and acyclovir, both alone and in combination. Acyclovir and interferon each produced dose-dependent decreases in virus titer at concentration ranges of 1 to 100 uM (approx 0.225 to 22.5 ug/ml) and 10 to 10,000 U/ml, respectively. Mean reductions in titer of 1.9 and 4.2 log10 PFU/ml were observed with 100 uM acyclovir and 10,000 U of interferon per ml, respectively, on day 10 of treatment. The combination of 100 uM acyclovir and 10,000 U of interferon per ml produced the most rapid fall in virus titer of all regimens examined and elimination of infectious virus by day 7. Prolonged treatment (greater than 10 days) with acyclovir or alpha interferon was accompanied by a gradual return of virus titer to control levels despite the continuous presence of drug. Virus preparations isolated from such cultures were tested for antiviral agent sensitivity by a plaque reduction method. Acyclovir-exposed isolates were found to be acyclovir resistant, with 50% inhibitory doses of greater than 200 uM, and to be thymidine kinase deficient. Alpha interferon-exposed isolates were not interferon resistant. These results suggest that, in persistently herpes simplex virus-infected CEM cells: (i)

combination treatment with 100 uM acyclovir and 10,000 U of alpha interferon per ml is more effective in reducing virus titer than either agent alone; (ii) prolonged exposure to drug may result in development of resistance by either the virus strain or the host cell system; and (iii) development of acyclovir resistance by herpes simplex virus in lymphoid cells is mediated by thymidine kinase. (Author abstract) (37 Refs)

51. ACTIVATION OF NON-EXPRESSED BOVINE PAPILLOMA VIRUS GENOMES BY TUMOUR PROMOTERS.

Amtmann E, Sauer G
Inst. Virus Res., German Cancer Res. Center, 6099 Heidelberg, W. Germany
Nature; 296(5858):675-677 1982

Following infection of mouse embryo fibroblasts (MEF), bovine papilloma virus type 1 (BPV-1) genome resides in a non-expressed episomal state. After passaging of the BPV-1 infected cells, the viral DNA is eventually lost due to failure to replicate, as shown by hybridization of the viral DNA to a BPV-1 DNA probe according to the Southern method. When BPV-1-infected MEF cells were treated with the tumor promotor 12-O-tetradecanoylphorbol-13-acetate (TPA), considerably more BPV-1 DNA was found after 4 days than in the non-treated cells. Even when TPA was administered to cells in the passage 3 after infection, viral DNA could be detected while none was detectable in non-treated passage 3 cells. Viral DNA was detected at passages 1 and 5 after TPA removal, indicating that BPV-1 DNA continues to replicate in the absence of TPA. TPA also promoted viral transcription. While no viral RNA sequences could be detected in untreated MEF cells one passage after infection, 4 days after TPA treatment of a parallel culture, several species of BPV-1 RNA were found. The same RNA species were detected in BPV-1-induced hamster embryo fibroblasts. Similar results to TPA were obtained with 12-O-retinoylphorbol-13-acetate (RPA). In addition to induction of viral transcripts, TPA induced a transformed phenotype, expressed by an increase in saturation density of the cultures and the ability to grow in low serum medium. While several primate cell lines showed none of these effects, bovine fetal thyroid (BFT) cells did exhibit an enhanced DNA replication and a transient transcription and phenotypic transformation in the presence of TPA and RPA. Tumor promoter-treated MEF and BFT cells may provide a system for studying the amplification and expression of foreign genes inserted into a BPV-1 vector as well as a screening system for putative tumor-promoting agents. (22 Refs)

52. INSERTION OF DRUG RESISTANCE GENES IN ANIMALS.

Bar-Eli M, Mercola KE, Slamon DJ, Mauritzson N, Stang HD, Cline MJ
(c/o Cline) Dept. Medicine, Center for Health Sciences, Univ. California at Los Angeles, Los Angeles, CA, 90024
J Cell Physiol; Suppl1:213-217 1982

Techniques for inserting new genetic information into animal cells, chromosome-mediated transfer of genetic information (CMTGI), microcell-mediated transfer, DNA-mediated transformation, direct injection of new genes, use of viral vectors to insert new genes, insertion of new genetic information into adult animals, methotrexate (MTX)-resistant dihydrofolate reductase (DHFR), inhibition by MTX, and polyacrylamide gel analysis are discussed. CMTGI technique utilizes isolated metaphase chromosomes as vectors for the transfer of genetic information. Microcells are single chromosomes or small groups of chromosomes contained within a nuclear membrane. Techniques also exist for isolating microcells from cells that grow in suspension culture rather than adherent to surface. A method utilizing cellular endocytosis of calcium microprecipitates of DNA has been used to insert highly purified DNA sequences containing specific genes. Genetic material can be injected directly into the nucleus of cells. Modified SV-40 has been used as a vector to introduce cloned genes into mammalian cells. Discrete portions of the SV-40 viral genome have been covalently linked to specific prokaryotic and eucaryotic genes and used in DNA-mediated transformation experiments. Experiments aimed at inserting new genes into the bone marrow cells of living mice based on previous studies in tissues culture were performed. Three mechanisms of resistance have been identified: mutations affecting intracellular transport of drug, mutations that alter the affinity of DHFR for MTX, and reduplication of presumably normal DHFR gene resulting in increased rates of enzyme synthesis and of cellular DHFR concentration. (42 Refs)

53. THE INFLUENCE OF ANTIVIRAL SERUM ON CELLS INFECTED WITH HERPES SIMPLEX VIRUS TYPE 1.

Karmysheva VYa, Rajcani J
Inst. Poliomyelitis and Viral Encephyalitides, USSR Acad. Medical Sciences, 142782 Moscow, USSR
Acta Virol (Praha); 26(1/2):79-85 1982

Homologous immune serum (IS) had an inhibitory effect on the accumulation of specific viral antigens in VERO cells infected with herpes simplex virus Type 1. When infected cells were incubated in the presence of IS, lower amounts of viral, both intracellular and membrane, antigens were found by quantitative immunofluorescence and immune cytolysis as compared to same infected cells incubated in the absence of IS. The possible mechanisms of this effect are discussed. (Author abstract) (15 Refs)

54. ULTRASTRUCTURAL STUDIES ON THE REPLICATION OF HERPES SIMPLEX VIRUS IN PK AND XTC-2 CELLS.

Ciampor F, Szanto J
Inst. Virology, Slovak Acad. Sciences, 809 39 Bratislava, Czechoslovakia
Acta Virol (Praha); 26(1/2):67-72 1982

Ultrastructural changes showed the following characteristics of restricted replication of herpes simplex virus 1 (HSV 1) strains MA and HSZP in PK and XTC-2 cells: (1) minimal cytopathic changes in PK cells as compared to more pronounced alterations in XTC-2 cells; (2) formation of single nucleocapsids or their absence in the nuclei of PK cells infected with the HSZP strain; (3) lack of budding and envelopment and absence of reduplication of the nuclear membrane; (4) persistence of partially uncoated virions within the endocytic vacuoles in the cytoplasm of PK cells; and (5) formation of dense inclusion bodies in addition to the presence of defective virions in the cytoplasm of XTC-2 cells and vacuolation of their cytoplasmic membranes. The replication of HsV 1 in PK and XTC-2 cells seemed to be blocked at both early and late stages of virus replication. At low multiplicity of infection, no virus particles were formed. (Author abstract) (14 Refs)

55. INITIAL STAGES IN INFECTION WITH ANIMAL VIRUSES.

Dimmock NJ
Dept. Biological Sciences, Univ. Warwick, Coventry CV4 7AL, England
J Gen Virol; 59(part1):1-22 1982

The initial stages of the interaction between animal viruses and their host cells, defined as virus-cell interactions which precede virus-directed synthetic processes, are reviewed. Attachment of virus to cellular receptors depends on the interaction of viruses with receptors on the cell surface and virus surface components which bind to cell receptors. Apparently, plasma proteins act as receptors, but because many of these are glycosylated determining whether the virus is binding to the polypeptide or carbohydrate moiety is difficult. Use of somatic cell fusion and isolation pf cell populations using a fluorescence-activated cell sorter are applied in the identification of receptors. Uptake of virus by the cell by translocation or direct penetration is the time when virus becomes integrated with the plasma membrane and passes through without fusing with it or becoming enveloped in a vesicle. Entry of a virus genome into a cell as a consequence of the fusion of virus and cell membranes is relevant only to enveloped viruses. Uptake of virus by the cell can also be performed by endocytosis. Uncoating may take place by fusion of enveloped viruses at the plasma membrane, follow endocytosis, or arise out of 'conformational changes' which cause the genome to become sufficiently permeable to allow replicative processes to commence. 'Conformational' uncoating is a destabilization of virion structure on infection which results from processes other than fusion or digestion by lysosomal enzymes and is relevant to the uncoating of picorna-, polyoma-, and adenoviruses. (215 Refs)

56. IDENTIFICATION OF DNA VIRUSES BY MEMBRANE FILTER HYBRIDIZATION.

Stalhandske P, Pettersson U
Dept. Microbiology, Biomedical Center, Univ. Upssala, S-751 23 Uppsala, Sweden
J Clin Microbiol; 15(4):744-747 1982

A procedure which allows DNA from a roller tube culture to be immobilized on nitrocellulose filters and the subsequent identification of the viral nucleic acids in the sample through molecular hybridization is described. Detection of herpes simplex virus (HSV) DNA in infected cells by hybridization is described and different methods for immobilizing DNA from infected cells are discussed. The time course of HSV infection is presented. Results indicate that it is possible to discriminate between HSV types 1 and 2 in a simple way. (15 Refs)

57. DIFFERENTIATION OF HERPES SIMPLEX VIRUS TYPES 1 AND 2 BY SENSITIVITY TO (E)-5-(2-BROMOVINYL)-2'-DEOXYURIDINE.

Mayo DR
Virology Lab., Veterans Admin. Medical Center, West Haven, CT, 06516
J Clin Microbiol; 15(4):733-736 1982

Use of (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) sensitivity as a marker was compared with a selective cell culture system which has been used routinely for typing herpes simplex virus (HSV) strains. Incorporation of BVDU directly into the culture media of cells inoculated with clinical specimens suspected of containing HSV resulted in a reduction of plaque formation in 23 HSV-1 isolates varying from 2.00-3.66 log10, while the plaque-forming ability of the HSV-2 strains was unaffected. BVDU used in this manner allowed for the presumptive identification of HSV types. (8 Refs)

58. METHYLATION OF THE VIRAL GENOME IN AN IN VITRO MODEL OF HERPES SIMPLEX VIRUS LATENCY.

Youssoufian H, Hammer SM, Hirsch MS, Mulder C
Dept. Pharmacology, Molecular Genetics and Microbiology, Univ. Massachusetts Medical Sch., Worcester, MA, 01605
Proc Natl Acad Sci USA; 79(7):2207-2210 1982

An in vitro model of latency of herpes simplex virus type 1 (HSV-1) in a lymphoid cell line has been developed recently. CEM cells persistently infected with HSV-1 transiently ceased to produce virus for 24 days. This nonproductive state could either be reversed with phytohemagglutinin or maintained with concanavalin A. This system was used to study the relationship between DNA methylation and HSV-1 latency. DNA was probed for methylation by comparing the cleavage patterns generated by two pairs of restriction endonucleases (Sma I vs Xma I and Hpa II vs Msp I); these enzymes show differential activity reflecting methylation of the recognition sequences. Viral DNA in the concanavalin A-treated cells (not producing virus) was found to be extensively methylated. By contrast, no methylated copies were detected in viral DNA from producer cells. About 800 days after the initial infection, the productive culture once again became nonproductive. Viral sequences in the latter cells were also methylated. Reconstitution experiments revealed 1-2 copies of viral DNA in cells from the latent stages and 40-80 copies in cells from productive stages. Most (if not all) of the viral genome is present in cells from various productive and latent stages. No differences in sequence arrangement were detected (although a terminal fragment of intracellular HSV-1 DNA appeared to be under-represented in latent cells). These results suggest a role for DNA methylation in the mechanism of HSV-1 latency in this system. (Author abstract) (23 Refs)

59. CLONING OF ADENO-ASSOCIATED VIRUS INTO PBR322: RESCUE OF INTACT VIRUS FROM THE RECOMBINANT PLASMID IN HUMAN CELLS.

Samulski RJ, Berns KI, Tan M, Muzyczka N
Dept. Immunology and Medical Microbiology, Univ. Florida Coll. Medicine, Gainesville, FL, 32610
Proc Natl Acad Sci USA; 79(6,part1):2077-2081 1982

We have cloned intact duplex adeno-associated virus (AAV) DNA into the bacterial plasmid pBR322. The AAV genome could be rescued from the recombinant plasmid by transfection of the plasmid DNA into human cells with adenovirus 5 as helper. The efficiency of rescue from the plasmid was sufficiently high to produce yields of AAV DNA comparable to those observed after transfection with equal amounts of purified virion DNA. Thus, the recombinant plasmid itself may be a model for studying the rescue of a latent AAV viral infection. In addition, the efficient rescue of viable AAV from the recombinant plasmid should facilitate the genetic analysis of AAV. Finally, the results of an analysis of the DNA from rescued virions indicate that an inversion of the AAV terminal sequences occurred during replication. (Author abstract) (19 Refs)

60. AMPLIFICATION OF EXPRESSION OF HEPATITIS B SURFACE ANTIGEN IN 3T3 CELLS COTRANSFECTED WITH A DOMINANT-ACTING GENE AND CLONED VIRAL DNA.

Christman JK, Gerber M, Price PM, Flordellis C, Edelman J, Acs G
Dept. Biochemistry and Pediatrics, Mount Sinai Medical Center, New York, NY, 10021
Proc Natl Acad Sci USA; 79(6,part1):1815-1819 1982

The 3T3 cells containing hepatitis B virus DNA sequences can be efficiently selected by exposure to methotrexate after cotransfection with cloned viral DNA and DNA coding for a methotrexate-resistant dihydrofolate reductase. More than 75% of methotrexate-resistant cells isolated after cotransfection with a head-to-tail tandem of the hepatitis B virus genome synthesized viral surface antigen. The antigen was released into the culture medium in the form of 22-nm particles with buoyant density of 1.20 g/ml. No other virally coded proteins were detected in the cells or the culture medium. Application of selective pressure by increasing the concentration of methotrexate resulted in an amplification of viral DNA sequences and a concomitant increase in the rate of synthesis and release of hepatitis B surface antigen appears to be a stable trait and has been maintained in these cultures through more than 30 passages. (Author abstract) (19 Refs)

61. ONCOGENIC VIRUS MEDIATED FORMATION OF TRANSFECTIVE DNA DURING ACUTE INFECTION OF CELLS WITH TICK-BORNE ENCEPHALITIS VIRUS.

Suetina IA, Parasiuk NA
D. I. Ivanovskii Inst. Virology, Moscow, USSR
Vopr Virusol; (1):45-48 1982

Formation of the transfective DNA was studied in cultured mutant cells of chinese hamsters and in cultured kidney cells of swine embryo. Cells were infected with tick-borne encephalitis virus (TBEV) and with simian virus 40 (SV40). Analysis of nucleic acid (NA) preparations isolated by fractionation in cesium sulfate gradient showed that the transfectivity was associated with NA fraction resistant to RNAse and sensitive to DNAse. (13 Refs) (In RUS)

62. HERPES SIMPLEX VIRUS: A CAUSE FOR CONCERN.

Alexander H
Dept. Lab. Medicine, Wesley Medical Center, Wichita, KS, 67214
Am J Med Technol; 48(4):241-245 1982

Laboratory facilities for the identification of herpes simplex virus (HSV) in tissue cultures should be available in every community. Evidence suggesting an association of genital HSV type 2 (HSV-2) infection with cervical carcinoma is primarily epidemiologic at present. Preliminary reports suggest that although topical treatment with acyclovir has little effect against recurrent HSV infections, systemic treatment of the initial infection significantly reduces the severity of the disease. However, this treatment does not eradicate latent HSV infections. (36 Refs)

63. SIGNIFICANT PROGRESS IN DERMATOLOGIC RESEARCH SINCE 1977.

Lapiere CM
Dept. Dermatology, Univ. Liege, B-4020 Liege, Belgium
J Am Acad Dermatol; 6(2):200-208 1982

Recent dermatologic research (1977-1981) of significance for cancer research includes: the identification of the Langerhans cells and partial elucidation of their role in cell-mediated immunity; the typing of mononuclear cells; the identification of T-lymphocyte growth factors; the association of defined subtypes of human papovavirus with neoplastic transformation; continuing research on melanogenesis and its control by polypeptides; the functions of specific proteins of the keratinocytes; the roles of membrane receptors, growth factors, and intracellular enzymes in the control of epidermal cell proliferation and differentiation; and phototherapy/photochemotherapy of cancer. (129 Refs)

64. ANTIVIRAL EFFECTS OF DEOXYPODOPHYLLOTOXIN ON HSV-INFECTED AMNION CELL CULTURES.

Markkanen T, Makinen ML, Miettinen J, Laijoki T, Maunuksela E, Hietanen T, Lehto I, Himanen P
Clinical Res. Lab., Univ. Turku, SF-20520 Turku 52, Finland
Drug Exp Clin Res; 8(1):27-34 1982

The effect of deoxypodophyllotoxin (DPT) on normal and herpes simplex-1 virus (HSV)-infected human amnion cell cultures was investigated. DPT sharply decreased the incorporation of thymidine, and moderately increased the incorporation of uridine and methionine in both normal and infected cells. The DPT-treated infected cultures showed no viral cytopathogenic effect. When DPT was removed by washing, incorporation of thymidine increased back to normal levels. DPT inhibited the proliferation of HSV, and did not exhibit virucidal activity. (14 Refs)

65. QUANTITATIVE STUDIES ON BLEOMYCIN-INDUCED STRAND BREAKAGE OF CHROMATIN DNA.

Kataoka K
Dept. Biochemistry, Cancer Inst., Okayama Univ. Medical Sch., Okayama 700, Japan
Okayama Igakkai Zasshi; 93(3/4):361-381 1981

Single and double strand breakage of DNA by bleomycin under various conditions was analyzed quantitatively using purified simian virus 40 (SV40) DNA and SV40 chromatin. The method employed for DNA assay was fluorometry of SV40 DNA separated into three forms I, II, and III by 1.4% agarose gel electrophoresis and stained with ethidium bromide. This method was extremely simple and accurate for quantitative estimation of single and double strand breakage of circular DNA. The amount of bleomycin required for in vitro breakage of SV40 chromatin DNA was much higher than that of naked SV40 DNA. 2-Mercaptoethanol accelerated bleomycin-induced DNA strand breakage. The concentration of 2-mercaptoethanol required for the acceleration was higher in SV40 chromatin than in SV40 DNA. DNA strand breakage was induced by ferrous ions in the absence of bleomycin to a similar level in SV40 chromatin and in SV40 DNA. The acceleration of bleomycin-induced strand breakage by a low concentration of ferrous ion was higher in SV40 DNA than in SV40 chromatin. Ethylenediamine tetraacetic acid (EDTA) and copper ions were not efficient for stopping the reaction of bleomycin-induced DNA breakage. Sodium dodecyl sulfate (SDS) was a useful reagent for stopping the reaction. (Author abstract) (75 Refs) (In JPN)

66. GENITAL INFECTION WITH HERPES SIMPLEX VIRUS.

Griffiths BL
Dept. Nursing, Univ. Alabama, Birmingham, AL
Nurs Times; 78(13):545-548 1982

The history and pathobiology of genital infection with herpes simplex virus (HSV) are discussed. HSV infection is most often accompanied by a history of sexual contact within the past 5-12 days. Recurrent disease is estimated to develop in 60-80% of patients who acquire the disease. HSV infection, while it is associated with an increased incidence of cervical cancer, should not be presented to the patient as a premalignant condition. Women with genital HSV should be encouraged to undergo regular cervical examination (every 6-18 mo). (4 Refs)

67. THE FUNCTIONS OF ATYPICAL LYMPHOCYTES.

Hutt-Fletcher LM
Dept. Bacteriology and Immunology, Univ. North Carolina, Chapel Hill, NC, 27514
The Handbook of Cancer Immunology Volume 6: Lymphoid Cell Subpopulations-Structure, Function, and Interactions. Waters H, ed. New York, Garland STPM Press, 314 pp., 1981.

Descriptive work on atypical lymphocytes (AL) is reviewed and morphology, metabolism, surface markers, and occurrence of AL are presented. AL in infectious mononucleosis (IM) and the role of the Epstein-Barr virus in IM are discussed, along with other causes of IM. In view the presence of AL in moderate numbers in certain neoplastic conditions, an understanding of their generation and capabilities may provide new approaches for immunotherapy in malignant disease. (79 Refs)

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DNA TUMOR VIRUSES IN NON-PRIMATE SYSTEMS

SERIES CB11 No. 82/10 OCTOBER, 1982

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VIRUS ISOLATION/ STRUCTURE/ CHARACTERIZATION/ IDENTIFICATION

1. TUMORIGENICITY OF HUMAN BK PAPOVAVIRUS PLAQUE ISOLATES, WILD-TYPE AND PLAQUE MORPHOLOGY MUTANT, IN HAMSTERS.

Watanabe S, Kotake S, Nozawa A, Muto T, Uchida S
Dept. Enteroviruses, NIH, Kamiosaki, Shinagawa-ku, Tokyo 141, Japan
Int J Cancer; 29(5):583-586 1982

The polyoncogenic prototype human papovavirus BKV (Gardner's strain) produced clear, small and large plaques. Two small-plaque isolates (wt-500 and wt-502), as well as a large-plaque-forming isolate (wt-501), induced frequent brain tumors and occasional osteosarcomas (but no insulinomas) in hamsters. The large-plaque former (wt-501) was more tumorigenic than the two small-plaque isolates. One small (pm-522) and one large turbid plaque mutant (pm-525), which had been rescued from hamster tumor cell lines and have small deletion near the origin of DNA replication, induced frequent brain tumors and insulinomas. Mutant 522 was about five times more tumorigenic than wt-501. These results support the hypothesis

HE 20.3173/2:

that the polyoncogenicity of prototype BKV is accounted for by the presence of BKV mutants. (Author abstract) (9 Refs)

2. BLA: A BURKITT LYMPHOMA-ASSOCIATED ANTIGEN DEFINED BY A MONOCLONAL ANTIBODY (MEETING ABSTRACT)

Lipinski M, Wiels J, Fellous M, Tursz T
Institut Gustave-Roussy, 94800 Villejuif, France
Fed Proc; 41(3):726 1982

A rat monoclonal IgM antibody (38-13) has been raised against Daudi cells, derived from a Burkitt lymphoma (BL). This antibody defined an antigen, referred to as BLA, specifically expressed on Burkitt tumor cells, whether they contain the Epstein-Barr virus (EBV) genome or not. BLA is not detected on EBV-positive lymphoblastoid cell lines, normal or mitogen activated lymphocytes, or fresh malignant cells from patients affected with various non-BL lymphoproliferative disorders. Therefore, 38-13 recognizes a BL-associated antigen clearly not related to EBV. Biochemical analysis indicates that BLA is a hydrophobic glycoconjugate: the antigen resists proteolytic treatments; no specific protein is detected by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) analysis after precipitation of radio-labeled BL membranes with 38-13; the antibody is specifically absorbed by a chloroform-methanol extract of BL cells; immunofluorescence staining for BLA is inhibited in the presence of D-galactose, N-acetylneuraminic acid and N-acetyl D-galactosamine. Since it displays an apparent specificity for BL cells, the 38-13 monoclonal antibody could represent a valuable tool for diagnosis and specific immunotherapy of BL. (no Refs)

3. ANALYSIS OF EPSTEIN-BARR VIRUS (EBV) OF P3HR-1: ISOLATION OF EBV WITH EBNA INDUCTION ABILITY IN HUMAN CORD LYMPHOCYTES AND WITHOUT EA INDUCTION ABILITY IN RAJI CELLS.

Yano S, Tanaka A, Takada K, Fujiwara S, Osato T, Nonoyama M
(c/o Nonoyama) Dept. Virology, Showa Univ. Res. Inst. Biomedicine Florida, 5180 113th Ave. N., Clearwater, FL, 33520
Virology; 119(2):392-398 1982

A subline of P3HR-1 cells was isolated through a prolonged (over 1 yr) propagation of the cells at a non-EBV-productive condition followed by cell cloning procedures. Cloned cells thus obtained, designated DHR1, produced EBV when brought back to the EBV-productive condition. Restriction enzyme analysis of the viral DNA revealed that DHR1 EBV is composed of an apparently homogeneous EBV population, and it displays a similar but not identical genome organization compared with HR-1 EBV. The characteristic biological properties of DHR1 EBV included the ability to induce EBV-associated nuclear antigen (EBNA) in human cord lymphocytes and the inability to induce EBV-associated early antigen (EA) in Raji cells. These are in striking contrast to the behavior of the parental HR-1 EBV. Thus, P3HR-1 cultures after a period of nonproductivity reinitiated the production of virus with an apparently homogeneous and unique population of EBV distinguishable from the original HR-1 virus. (Author abstract) (18 Refs)

VIRUS ASSOCIATED CELL TRANSFORMATION AND HOST ALTERATION

4. ROLE OF VIRUSES IN THE INDUCTION OF PRIMARY INTRACRANIAL TUMORS.

Walsh JW, Zimmer SG, Perdue ML
Div. Neurosurgery, Univ. Kentucky Medical Center, 800 Rose St., Lexington, KY, 40536
Neurosurgery; 10(5):643-662 1982

The literature on viral induction of intracranial tumors is reviewed, with special emphasis on the papovaviruses of the simian virus (SV)40 group. Discussion includes oncogenic studies in vivo and in vitro, the molecular basis for infection and transformation, and evidence that the papovavirus may cause human intracranial tumors. The latter was partly obtained from studies on children inoculated with live polio virus contaminated with SV40 who developed an excess of tumors, including intracranial tumors. (188 Refs)

5. ENHANCING ELEMENTS FOR ACTIVATION OF EUKARYOTIC PROMOTERS.

Yaniv M
Institut Pasteur, 28 rue du Dr Roux, 75724 Paris Cedex 15, France
Nature; 297(5861):17-18 1982

Short viral sequences that stimulate the transcription of coding sequences from their own promoters, termed 'enhancers,' are discussed. Viral enhancers can affect the activity of eukaryotic genes; they are not limited to papovavirus and have been found in retroviruses. The presence of enhancers in retroviruses may explain results that do not fit the simple promotion model of viruses as applied to oncogenesis. Enhancers may play a role in regulating cellular differentiation at the level of tissue- or cell type-specific control of gene expression. (22 Refs)

6. CELLULAR PROTEINS REACTIVE WITH MONOCLONAL ANTIBODIES DIRECTED AGAINST SIMIAN VIRUS 40 T-ANTIGEN.

Crawford L, Leppard K, Lane D, Harlow E
Dept. Molecular Virology, Imperial Cancer Res. Fund Lab., Lincoln's Inn Fields, London WC2A 3PX, England
J Virol; 42(2):612-620 1982

Several recently isolated monoclonal antibodies which reacted with simian virus 40 T antigens also reacted with proteins found in uninfected and untransformed cells. The proteins were different from each other, PAb419 reacting with a 35,000-mol-wt protein, PAb427 reacting with a 75,000-mol wt phosphoprotein, PAb405 reacting with a 150,000-mol-wt phosphoprotein, and PAb204 reacting with a 68,000-mol wt protein. It is suggested that although some of these cross-reactions may be fortuitous, they may, as an alternative, reflect similarities of shape and perhaps function between domains of the viral T antigen and the relevant host proteins. (Author abstract) (29 Refs)

7. PRODUCTION OF A T-ANTIGEN-RELATED PROTEIN IN MAMMALIAN CELLS AFTER STABLE TRANSFORMATION WITH A CLONED SV40 GENE FRAGMENT.

Horst J, Jacob E, Weckler C, Deppert W
Abteilung Humangenetik, Universitat Ulm, Oberer Eselsberg, D-7900 Ulm, W. Germany
Hoppe Seylers Z Physiol Chem; 363(4):445-448 1982

A recombinant plasmid based on pBR322 has been constructed which carries the replicator proximal early region of SV40 DNA, including the viral origin of replication (ORI). It lacks a major part of the tumor antigen 3'-coding region, the large T-antigen termination codon and the polyadenylation site. The recombinant plasmid was transferred together with the herpes simplex virus thymidine kinase gene, as a selectable marker into mouse LTK- cells. Integration and expression of the cloned SV40 gene fragment in TK+ transformants could be demonstrated by DNA restriction and blot hybridization and by immunofluorescence techniques. (Author abstract) (12 Refs)

8. MUTANT OF SIMIAN VIRUS 40 LARGE T-ANTIGEN THAT IS DEFECTIVE FOR VIRAL DNA SYNTHESIS, BUT COMPETENT FOR TRANSFORMATION OF CULTURED RAT CELLS.

Stringer JR
Cold Spring Harbor Lab., Cold Spring Harbor, NY, 11724
J Virol; 42(3):854-864 1982

A mutant was isolated which demonstrates that the transforming activity of simian virus 40 large T-antigen is separable from its function in viral DNA replication. The mutant, SVR9D, is nonconditionally defective for viral DNA synthesis, but competent at wild-type level for morphological transformation of cultured rat cells. The lytic growth defect in SVR9D is complemented by the simian virus 40 A gene product present in the transformed CV1 cell line, COS1. The lesion in SVR9D DNA was mapped genetically by marker rescue of plaque formation and localized to a 214-base-pair segment of the viral genome bounded by nucleotide numbers 4100 and 4314. DNA sequence analysis showed the mutation to be an adenine-to-guanine transition at nucleotide number 4178. This change predicts a lysine-to-glutamic acid amino acid change at residue number 214 of the mutant large T-antigen polypeptide. (Author abstract) (32 Refs)

9. PRESENCE IN GROWTH-ARRESTED CELLS OF CELLULAR PROTEINS THAT INTERACT WITH SIMIAN VIRUS 40 SMALL-T ANTIGEN.

Rundell K
Dept. Microbiology and Immunology, Northwestern Univ., Chicago, IL, 60611
J Virol; 42(3):1135-1137 1982

Two cellular proteins, 56K and 32K, found in association with simian virus 40 small-t antigen were not induced by viral infection. In addition, the proteins were expressed by cells in the growth arrest period, a time in which small-t function is of importance in infection and transformation. (Author abstract) (12 Refs)

10. POLYOMA VIRAL MIDDLE T-ANTIGEN IS REQUIRED FOR TRANSFORMATION.

Mes AM, Hassel JA
(c/o Hassel) Dept. Microbiology and Immunology, McGill Univ., Montreal, Quebec H3A 2B4, Canada
J Virol; 42(2):621-629 1982

To determine whether small or middle T-antigen (or both) of polyoma virus is required for transformation, we constructed mutants of recombinant plasmids which bear the viral oncogene and measured the capacity of these mutants to transform rat cells in culture. Insertion and deletion mutations in sequences encoding small and middle T-antigens (79.7, 81.3, and 82.9 map units) rendered the DNA incapable of causing transformation by the focus assay. Similar mutations in sequences that encoded middle but not small T-antigen (89.7, 92.1, and 96.5 map units) generally abolished the transforming activity of the DNA. However, two mutants (pPdl1-4 and pPdl2-7) that carried deletions of 92.1 map units retained the capacity to transform cells; pPdl1-4 did so at frequencies equal to those of the parental plasmid, whereas pPdl2-7 transformed at 10% the frequency of its antecedent. From these studies we conclude that small T-antigen alone is insufficient to cause transformation and that middle T-antigen is required for transformation, either in combination with small T-antigen or by itself. (Author abstract) (43 Refs)

11. ISOLATION OF TRANSFORMATION-DEFECTIVE HOST-RANGE MUTANTS OF POLYOMA VIRUS ON NORMAL MOUSE CELLS.

Feunteun J, Benjamin TL
Institut de Recherches Scientifiques sur le Cancer, BP No. 8, 94802 Villejuif Cedex, France
Virology; 119(2):310-316 1982

Host-range mutants of polyoma virus have been isolated utilizing primary mouse embryo fibroblasts as the permissive host and cells of an established 3T3 line as the nonpermissive host. Five out of five such 'mouse embryo-dependent' (MED) mutants have totally lost transforming ability. Their transformation defects can be complemented by ts-a mutants but not by hr-t mutants. Restriction enzyme digestion shows deletions of 150-200 base pairs in the proximal early region of the viral DNAs (HpaII fragment 4, 79-92 map units). None of the MED mutants produce middle or small tumor antigen species detectable by metabolic labeling and immune precipitation, and none show the middle T-antigen-associated protein kinase activity in vitro. MED mutants are thus indistinguishable from hr-t mutants previously isolated ising polyoma-transformed 3T3 cells as the permissive host. The isolation of nontransforming host-range mutants in the absence of direct polyoma gene complementation confirms a specific prediction of the hypothesis of indirect action by the hr-t gene, according to which the inability to transform must arise concomitantly with the inability to induce a permissive state for virus growth. (Author abstract) (28 Refs)

12. HERPESVIRUS INFECTION ALTERS THE STEADY-STATE LEVELS OF CELLULAR POLYADENYLATED RNA IN POLYOMA VIRUS-TRANSFORMED BHK CELLS.

Nakai H, Maxwell IH, Pizer LI
(c/o Pizer) Dept. Microbiology/Immunology, Univ. Colorado Sch. Medicine, Denver, CO, 80262
J Virol; 42(3):1131-1134 1982

Polyoma-transformed BHK cells are permissive for the replication of herpes simplex virus Type 1. The effect of herpes in-

fection on the steady-state levels of bulk mRNA sequences in these cells was studied by using cDNA to polyadenylated cytoplasmic RNA from uninfected cells. The principal findings were: (i) herpes simplex virus Type 1 infection caused a pronounced reduction in the cytoplasmic levels of moderately abundant mRNAs; (ii) after infection, increased amounts of RNA complementary to the cDNA were isolated as part of the nonadenylated cytoplasmic RNA fraction. (Author abstract) (14 Refs)

13. CHANGE OF DNA NEAR THE ORIGIN OF REPLICATION ENHANCES THE TRANSFORMING CAPACITY OF HUMAN PAPOVAVIRUS BK.

Watanabe S, Yoshiike K
Dept. Enteroviruses, Natl. Inst. Health, Kamiosaki, Shinagawa-ku, Tokyo 141, Japan
J Virol; 42(3):978-985 1982

A turbid-plaque-forming mutant (pm522) of human papovavirus BK, which has a small deletion at about 0.7 map unit and grows somewhat more slowly in human cells than does wild-type BK virus, transformed hamster and rat cells in culture much more efficiently than did wild-type virus. Another plaque morphology mutant, pm525, forming turbid plaques larger than those of pm522 also had a high transforming capacity. The similar difference in transforming capability between wild-type and plaque morphology viruses was observed with DNAs extracted from virions. Recombinant viruses were constructed from the wild-type DNA fragment lacking HindIII-C (0.62 to 0.73 map unit) and pm522 HindIII-C (including the origin of replication) by the molecular cloning method. Characterization of the recombinants showed that the change near the origin of DNA replication was responsible both for the altered plaque morphology and for the enhanced transforming capacity of the BK virus mutant. (Author abstract) (33 Refs)

14. DEPENDENCE OF TUMOR-FORMING CAPACITIES OF CELLS TRANSFORMED BY RECOMBINANTS BETWEEN ADENOVIRUS TYPES 5 AND 12 ON EXPRESSION OF EARLY REGION 1.

Shiroki K, Maruyama K, Saito I, Fukui Y, Yazaki K, Shimojo H
(c/o Shimojo) Inst. Medical Science, Univ. Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo, 108, Japan
J Virol; 42(2):708-718 1982

Recombinants between an adenovirus Type 5 (Ad5) deletion mutant and the Ad12 DNA fragment containing early Region 1 (E1) were isolated from cells cotransfected with the EcoRI-C fragments of Ad12DNA and Ad5 dl312 (deletion in E1A) DNA (rcA) and from cells cotransfected with the SalI-C fragment of Ad12 DNA and Ad5 dl312 DNA (rcB). No recombinant was isolated from cells cotransfected with Ad5 dl313 (deletion in E1B) DNA and restriction fragments of Ad12 DNA. Both rcA and rcB are defective and able to replicate in human embryo kidney (HEK) and KB cells with complementation by dl312. Both rcA and rcB formed Ad12 T antigen g, but not T antigen f, in infected HEK and KB cells. In rcA- and rcB-infected cells, Ad5 E1B and Ad12 E1A genes are transcribed. Heteroduplex and size analysis of rcA-1 or rcB-1 DNA fragments hybridized with Ad12 DNA revealed that rcA-1 DNA has a deletion between 5 and 15 map units with an insertion of a portion of Ad12 DNA (10%) and that rcB-1 DNA has a deletion between 70 and 80 map units with an insertion of a portion of Ad12 DNA (10%). The transformed cell lines, RCAY and RCBY, were established after infection of rat 3Y1 cells with rcA and rcB, respectively. Both Ad5 and Ad12 DNA sequences are contained in these cells. In RCAY cells, Ad12 T antigen g is detected, but Ad12 T antigen f is not. In RCBY cells, both Ad12 T antigens g and f are detected. Only the Ad12 E1A gene is transcribed in RCAY cells, whereas Ad5 E1B, Ad12 E1A, and Ad12 E1B genes are transcribed in RCBY cells. In soft-agar cultures, RCBY cells form large colonies, whereas RCAY cells form only tiny colonies. RCBY cells form tumors as efficiently as 12WY cells in transplanted rats. RCAY cells formed tumors inefficiently. Ad5-transformed 5WY cells do not form tumors. These observations indicate that the efficient tumor formation by RCBY cells is dependent on the expression of the Ad12 E1A and E1B genes, whereas the inefficient tumor formation by RCAY cells is due to the expression of only the Ad12 E1A gene. (Author abstract) (28 Refs)

15. SENSITIVITY OF ADENOVIRUS-TRANSFORMED CELL LINES OF DIFFERENT TUMORIGENICITY TO KILLING BY SYNGENEIC NATURAL KILLER CELLS (MEETING ABSTRACT)

Raska K, Gallimore PH
Department of Pathology, CMDNJ-Rutgers Medical School, Piscataway, NJ, 08854
Proc Am Assoc Cancer Res; 23:949 1982

In this study, the tumorigenic potentials of six adenovirus-transformed LIS cell lines were studied in syngeneic rats. The cells used are well characterized as to the content and expression of the adenovirus genome. The in vivo tumorigenic potential of these individual cell lines in LIS rats varied from nontumorigenicity in immunosuppressed newborn animals to high tumorigenicity in untreated newborn and weanling rats. This different capacity to induce tumors does not correlate with a number of in vitro properties of these cell lines, such as growth saturation density, decreased doubling time, increased fibrinolytic activity or anchorage independent growth. Similarly, the ability of these cell lines to elicit the cytolytic T cell response in syngeneic LIS rats also does not universally correlate with their tumorigenic potential. When the capacity to induce tumors in vivo, however, was compared with the sensitivity of these transformed cell lines to in vitro killing by natural killer (NK) cells in syngeneic lymphoid cell preparations, an inverse relation was found between these two parameters. In parallel experiments no significant differences were detected in the sensitivity of these cell lines to in vitro killing with syngeneic secondary cytolytic T cells. (no Refs)

16. PURIFICATION OF A NATIVE MEMBRANE-ASSOCIATED ADENOVIRUS TUMOR ANTIGEN.

Persson H, Katze MG, Philipson L
(c/o Katze) Molecular Biology and Genetics Unit, Graduate Sch. Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
J Virol; 42(3):905-917 1982

A 15,000-dalton protein was purified from HeLa cells infected with adenovirus Type 2. Proteins solubilized from a membrane fraction of lytically infected cells was used as the starting material for purification. Subsequent purification steps involved lentil-lectin, phosphocellulose, hydroxyapatite, DEAE-cellulose, and aminohexyl-Sepharose chromatographies. A monospecific antiserum, raised against the purified protein, immunoprecipitated a 15,000-dalton protein encoded in early-region E1B (E1B/15K protein) of the adenovirus Type 2 DNA. Tryptic finger print analysis revealed that the purified protein was identical to the E1B/15K protein encoded in the transforming part of the viral genome. The antiserum immunoprecipitated the E1B/15K protein from a variety of viral trans-

formed cell lines isolated from humans, rats, or hamsters. The E1B/15K protein was associated with the membrane fraction of both lytically and virus-transformed cell lines and could only be released by detergent treatment. Furthermore, an 11,000-to 12,000-dalton protein that could be precipitated with the anti-E1B/15K serum was recovered from membranes treated with trypsin or proteinase K, suggesting that a major part of the E1B/15K protein is protected in membrane vesicles. Translation of early viral mRNA in a cell-free system, supplemented with rough microsomes, showed that this protein was associated with the membrane fraction also in vitro. (Author abstract) (47 Refs)

17. EPSTEIN-BARR VIRUS (EBV) GENOME IN HUMAN LYMPHOMA CELLS AMPLIFIES EXPRESSION OF THE PROVIRAL MURINE LEUKEMIA VIRUS (MULV) P30 (MEETING ABSTRACT)

Lasky RD, Troy FA
Department of Biol Chem, University of California School of Medicine, Davis, CA, 95616
Fed Proc; 41(3):1387 1982

EBV-negative lymphoma lines, Ramos and BJAB, can be converted in vitro to EBV-positive sublines thus providing a uniclonal tumor cell population in which the same line can be compared with and without the EBV genome. We have used this system to distinguish between trivial subline difference and differences related to the presence of the EBV genome. The antigenic profiles of plasma membranes were examined using sera from immunized rabbits. Tumor cells proteins were resolved by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and then electrophoretically transferred to nitrocellulose paper. Anti-tumor cell sera was allowed to bind to the antigens. Binding was detected using 125I-labeled antibodies to the IgG of the primary sera, followed by autoradiography. Electro-blotting revealed several distinct differences. A major difference was an increase in a 30K dalton protein (p30) in the Ramos converted sublines. In contrast, no p30 seen in BJAB or its converted lines. When Ramos, but not BJAB, was originally established in culture, it was passaged in athymic nude mice and acquired an MuLV provirus. Present results suggested that the EBV-genome in Ramos converted cells amplified expression of the mouse p30. This conclusion was confirmed using antisera specific for MuLV and p30. How EBV is promoting enhancement of retroviral proteins in these human lymphoma cells and its possible biological significance is not clear. (no Refs)

18. A MURINE MODEL SYSTEM FOR STUDYING THE MOLECULAR MECHANISM OF EPSTEIN-BARR VIRUS (EBV)-MEDIATED CELL TRANSFORMATION AND CARCINOGENESIS (MEETING ABSTRACT)

Volsky DJ, Shapiro IM, Kuszynski C
Department of Pathology, Eppley Cancer Institute, University of Nebraska Medical Center, Omaha, NE, 68105
Fed Proc; 41(3):688 1982

EBV can normally infect in vitro only human and marmoset B lymphocytes, leading to cell transformation without any primary viral reproductive cycle. Using our method of EBV receptor-implantation we demonstrated that any other cell which is artificially provided with EBV receptors can be infected by the virus. Here we show also that EBV receptor-implanted mouse lymphocytes are infectible by EBV, when using either the transforming B-95-8 (B-EBV) or non-transforming P3HR-1 (P-EBV) substrains of the virus. Unlike the natural host, mouse lymphocytes are not readily transformed by B-EBV. Instead, the cells support a full viral lytic cycle, resulting in the release of a new biologically active EBV isolate (M-EBV). Unlike the natural host, the EBV-infected mouse cells express the virus-determined early (EA) and virus capsid (VCA) antigens. Suprisingly, the EBV-nuclear antigen (EBNA) is barely detectable. This model system is used to test the hypothesis that the EBV-related oncogenic mechanism involves a virus-mediated increase in intracellular proteins, presumably as a complex between a virus coded 48,000 dalton component and a cell-coded 53,000 dalton component, which may block the viral lytic cycle and interfere with cell differentiation leading to transformation. (no Refs)

19. IDENTIFICATION OF PROTEINS ENCODED BY A FRAGMENT OF HERPES SIMPLEX VIRUS TYPE 2 DNA THAT HAS TRANSFORMING ACTIVITY.

Galloway DA, Goldstein LC, Lewis JB
Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
J Virol; 42(2):530-537 1982

Cloned BglII fragment N (map units 0.58 to 0.625) of herpes simplex virus Type 2 DNA has been shown to transform rodent cells to an oncogenic phenotype. RNA homologous to this fragment directs the synthesis of five polypeptides in a cell-free translation system. The approx mol wt of these proteins are 140,000, 61,000, 56,000, 35,000, and 23,500. The 35,000-dalton protein is the major species late in infection and is the only species detected before the onset of viral DNA replication. The arrangement of the sequences encoding these proteins along the herpes simplex virus Type 2 genome was determined by hybridization of the RNA to cloned PstI fragments of BglII-N and to single-stranded DNA segments cloned into M13mp7. Both the hybridization experiments and immunoprecipitation with monoclonal antibodies suggested that the 140,000-and 35,000-dalton proteins are at least partially colinear and share antigenic determinants. (Author abstract) (41 Refs)

20. A HETEROANTISERUM AGAINST AMERICAN BURKITT'S LYMPHOMA (BL) (MEETING ABSTRACT)

Magrath IT, Parsons RG
Pediatric Oncology Branch, National Cancer Institute, Bethesda, MD, 20205
Proc Am Assoc Cancer Res; 23:1039 1982

We have raised a goat antiserum (B75) against purified membrane from an American BL cell line and examined its spectrum of reactivity after extensive absorption with either 5 African BL cell lines or 6 Epstein Barr Virus-transformed cord blood lymphocyte (CBL) cell lines. Both methods of absorption yielded antisera with identical specificities. Eleven of 12 EBV genome-negative cell lines derived from American BL (6) and undifferentiated non-BL (6) reacted with B75 using indirect immunofluorescence. Three of 4 EBV genome-positive American undifferentiated lymphomas were also positive, but none of 13 CBL lines or 8 non-tumor lymphoblastoid cell lines reacted with B75. Two of 7 African BL cell lines reacted positively. These 2 lines also secreted small amounts of IgM, whereas none of the 5 negative lines did so. B75 did react with blast cells derived from common acute lymphoblastic leukemia (ALL) but not with blast cells from T-cell ALL or lymphoblastic lymphoma. B75 reactivity persists after incubation of cells with proteolytic enzymes and we have so far been unable to immunoprecipitate a protein from cell lysates with B75. These data indicate that BL cells express one or more antigens not present on other lymphoid cell lines of normal and neoplas-

tic origin. These data are consistent with the hypothesis that American-BL and undifferentiated lymphoma are the neoplastic counterparts of cell types early in the B-cell pathway, probably immediately after the pre-B cell stage, and before the normal counterpart of African BL. (no Refs)

21. CHEMICAL INTERACTIONS WITH HERPES SIMPLEX TYPE 2 VIRUS: ENHANCEMENT OF TRANSFORMATION BY HYDRAZINE AND 1,2-DIMETHYLHYDRAZINE.

Johnson FB
Dept. Microbiology, Brigham Young Univ., Provo, UT, 84602
Chem Biol Interact; 40(1):97-112 1982

Quantitative assays for the morphological transformation of 3T3 Swiss mouse cells by herpes simplex Type 2 virus (HSV-2) were employed to examine the effect on cell transformation of chemical carcinogens and suspected carcinogens. Exposure of the cells to the chemical compound, followed by virus infection, resulted in enhancement of transformation when compared to that observed with chemical or virus alone. Enhancement occurred in tests utilizing either UV light-inactivated HSV-2 (strain 333) or a temperature-sensitive (ts) mutant of HSV-2 [A8(293)]. A series of seven ts-mutants were tested and exhibited varying degrees of transformation. Enhancement of transformation occurred in cells treated with hydrazine (HZ) and 1,2-dimethylhydrazine (SDMH). No enhancement occurred when cells were treated with monomethylhydrazine, 1,1-dimethylhydrazine and the jet fuels JP-5, JP-10, RJ-4 and RJ-5. A strong time dependence after treatment was demonstrated with some enhancement seen at 6 hr after chemical treatment but the greatest enhancement appeared when virus infection began after 24 hr of chemical exposure. (Author abstract) (25 Refs)

22. ALTERATIONS IN PROTEIN AND GLYCOPROTEIN COMPOSITION OF RAT EMBRYO FIBROBLASTS TRANSFORMED BY HERPES SIMPLEX VIRUS TYPE 2.

Rossowski W, Blaszczyk M, Darai G, Munk K
Inst. Virus Res., German Cancer Res. Center, D-6900 Heidelberg, W. Germany
Oncology; 39(4):222-227 1982

Total and superficially exposed plasma membrane components of tumorigenic herpes-simplex-virus(HSV)-transformed fibroblasts were studied. As a result of oncogenic HSV transformation, a significant decrease of polypeptides 230,000, 180,000, 56,000 and 43,000 daltons was found. These changes were accompanied by a significant increase in superficial exposition of several sialopeptides and two peptides of 30,000 and 15,000 daltons. It was suggested that these two peptides may represent virus-coded components of HSV-transformed fibroblasts. The present results indicate that the HSV-transformed tumorigenic cell clone meets all previously described criteria for oncogenically transformed cells. (Author abstract) (38 Refs)

23. CHEMICAL INTERACTIONS WITH HERPES SIMPLEX TYPE 2 VIRUS: ENHANCEMENT OF TRANSFORMATION BY NORHARMAN.

Johnson FB
Dept. Microbiology, Brigham Young Univ., Provo, UT, 84602
Carcinogenesis; 3(4):457-459 1982

The effects of the comutagen norharman on herpes simplex virus Type 2 growth transformation was studied in 3T3 cells. Pre-exposure but not post-exposure of the cells to 0.5 to 10 ug of norharman per ml of cell culture media resulted in a nearly three-fold enhancement in transforming activity. These results suggest that certain environmental chemicals may interact with cells creating an increased possibility of transformation by herpes viruses. (Author abstract) (27 Refs)

VIRUS REPLICATION AND EXPRESSION

24. EXPRESSION OF ADENOVIRAL GENETIC INFORMATION IN PRODUCTIVELY INFECTED CELLS.

Flint SJ
Dept. Biochemical Sciences, Princeton Univ., Princeton, NJ, 08544
Biochim Biophys Acta; 651(2/3):175-208 1982

Recent advances in the understanding of mechanisms and regulation of adenoviral gene expression are reviewed. Topics discussed include the structure of adenoviral early genes (early regions E1-E4 and expression of additional genes during early phase of infection), synthesis of adenoviral early messenger RNA species, regulation of adenoviral early gene expression, organization and expression of adenoviral late genes, the early to late transition, structure and function of adenoviral 'promoter' sites, and the virus associated (VA)-RNA genes. (225 Refs)

25. MECHANISM OF ACTION OF DICHLORO-BETA-D-RIBOFURANOSYLBENZIMIDAZOLE: EFFECT ON IN VITRO TRANSCRIPTION.

Zandomeni R, Mittleman B, Bunick D, Ackerman S, Weinmann R
Wistar Inst. Anatomy and Biology, 36th and Spruce St., Philadelphia, PA, 19104
Proc Natl Acad Sci USA; 79(10,part1):3167-3170 1982

The adenosine analog 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole (DRB) and its mono- and triphosphate derivatives inhibit RNA polymerase II-specific transcription in an extract of whole HeLa cells. The analog does not inhibit RNA polymerase III-specific adenovirus VA RNA transcription in the whole cell extract. With purified RNA polymerase II under nonspecific transcription conditions, no effect of DRB could be detected. DRB is equally effective in inhibiting in vitro transcription from several of the adenovirus promoters and the human epsilon-globin gene. The inhibitory effects are in the order DRB greater than DRB monophosphate greater than DRB triphosphate. Thus DRB acts in vitro presumably on systems in which specific RNA polymerase II initiation of transcription occurs and with no detectable effect on premature termination. This will provide a suitable model for study of the molecular mechanism of action of DRB on transcription. (Author abstract) (24 Refs)

26. EXPRESSION OF THE GENE ENCODING THE ADENOVIRUS DNA TERMINAL PROTEIN PRECURSOR IN PRODUCTIVELY INFECTED AND TRANSFORMED CELLS.

Binger MH, Flint SJ, Rekosh DM
Dept. Biochemical Sciences, Princeton Univ., Princeton, NJ, 08544
J Virol; 42(2):488-501 1982

The major product of in vitro translation of early RNA prepared from H5ts125-infected cells and selected by hybridization to adenoviral DNA fragments spanning the region from 14.7 to 31.5 map units has been shown to be identical to the 87-kilodalton terminal protein precursor. A 72-to 75-kilodalton po-

lypeptide whose mRNA can be selected by DNA from this region and made in the presence of anisomycin was indistinguishable from the 72-kilodalton single-stranded DNA-binding protein encoded by the region from 60.1 to 66.6 map units. The accumulation of cytoplasmic RNA sequences complementary to these l-strand genes under various conditions of infection and in certain lines of transformed cells has been investigated by solution hybridization of cytoplasmic RNA to the separated strands of restriction endonuclease fragments of adenoviral DNA. During the early phase, RNA sequences complementary to the region from 11.6 to 36.7 map units were present at a concentration of 10 to 60 copies per cell, regardless of the nature of the block used to inhibit viral DNA synthesis. By 24 hr after infection in the absence of any such block, sequences complementary to the regions from 11.6 to 18.2 map units (IVa2) and from 18.6 to 36.7 map units (E2B) accumulated to concentrations of 4,800 and 280 copies per cell, respectively. The ratio of cytoplasmic E2A RNA sequences to E2B RNA sequences remained close to 10:1 throughout the time period investigated. Of the transformed cell lines which retained E2B DNA sequences that were examined, only the T2C4 line expressed these sequences in cytoplasnic RNA. The implications of these observations for regulation of expression of the adenoviral early l-strand genes are discussed. (Author abstract) (73 Refs)

27. AN ADENOVIRUS AGNOGENE.

Virtanen A, Alestrom P, Persson H, Katze MG, Pettersson U
Dept. Microbiology, Biomedical Center, Univ. Uppsala, Box 581, S-751 23 Uppsala, Sweden
Nucleic Acids Res; 10(8):2539-2548 1982

The nucleotide sequence of a 550 base pairs long segment, located between map positions 21 and 22.5 in the adenovirus Type 2 genome has been determined. S1 nuclease mapping and sequence analysis of cDNA copies of adenovirus mRNA demonstrated that the established sequence includes the i-leader which is spliced to the 5'-end of certain adenovirus mRNAs. The i-leader which is 440 nucleotides long contains an open transitional reading frame which is preceded by an AUG triplet and which terminates in the third segment of the tripartite leader. A polypeptide with the same mol wt as predicted from the DNA sequence was identified by in vitro translation of mRNA which had been selected by hybridization to DNA fragments, containing sequences from the i-leader. The results thus suggest that the i-leader, unlike other adenovirus leader segments, is used for translation. (Author abstract) (27 Refs)

28. SITE OF PREMATURE TERMINATION OF LATE TRANSCRIPTION OF SIMIAN VIRUS 40 DNA: ENHANCEMENT BY 5,6-DICHLORO-1-BETA-D-RIBOFURANOSYLBENZIMIDAZOLE.

Skolnik-David H, Hay N, Aloni Y
Dept. Genetics, Weizmann Inst. Science, Rehovot 76100, Israel
Proc Natl Acad Sci USA; 79(9):2743-2747 1982

Sedimentation analysis of pulse-labeled RNA synthesized in nuclei isolated from simian virus 40-infected cells revealed an abundance of short cellular and viral RNAs. The reactive amount of the short chains is increased in nuclei isolated from cells treated with 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole (DRB). The short viral RNAs were purified by hybridization to and elution from simian virus 40 DNA on filters, and their sizes were determined by gel electrophoresis. A major band of 93-to 95-nucleotide-long RNA was observed along with additional minor bands. Identical bands were revealed when the viral RNA was purified from nuclei of cells pretreated with DRB. The major band was identified as an aborted transcript of a RNA that initiated at the major initiation site (nucleotide 243). We have found that the DNA region where the RNA stops is A + T rich and is immediately preceded by a G + C-rich region that exhibits dyad symmetry, resembling the termination signal in prokaryotes. These observations show that RNA polymerase II responds to the same termination signal as the prokaryotic enzyme and suggest that a mechanism of attenuation regulates simian virus 40 late transcription. (Author abstract) (47 Refs)

29. RECOGNITION SITES OF EUKARYOTIC DNA TOPOISOMERASE I: DNA NUCLEOTIDE SEQUENCING ANALYSIS OF TOPO I CLEAVAGE SITES ON SV40 DNA.

Edwards KA, Halligan BD, Davis JL, Nivera NL, Liu LF
(c/o Liu) Dept. Physiological Chemistry, Johns Hopkins Medical Sch., Baltimore, MD, 21205
Nucleic Acids Res; 10(8):2565-2576 1982

Eukaryotic DNA topoisomerase I introduces transient single-stranded breaks on double-stranded DNA and spontaneously breaks down single-stranded DNA. The cleavage sites on both single and double-stranded SV40 DNA have been determined by DNA sequencing. Consistent with other reports, the eukaryotic enzymes, in contrast to prokaryotic Type I topoisomerases, links to the 3'-end of the cleaved DNA and generates a free 5'-hydroxyl end on the other half of the broken DNA strand. Both human and calf enzymes cleave SV40 DNA at identical and specific sites. From 827 nucleotides sequenced, 68 cleavage sites were mapped. The majority of the cleavage sites were present on both double and single-stranded DNA at exactly the same nucleotide positions, suggesting that the DNA sequence is essential for enzyme recognition. By analyzing all the cleavage sequences, certain nucleotides are found to be less favored at the cleavage sites. There is a high probability to exclude G from positions-4,-2,-1 and +1, T from position-3, and A from position-1. These five positions (-4 to +1 oriented in the 5' to 3' direction) around the cleavage sites must interact intimately with topo I and thus are essential for enzyme recognition. One topo I cleavage site which shows an atypical cleavage sequence maps in the middle of a palindromic sequence near the origin of SV40 DNA replication. It occurs only on single-stranded SV40 DNA, suggesting that the DNA hairpin can alter the cleavage specificity. The strongest cleavage site maps near the origin of SV40 DNA replication at nucleotide 31-32 and has a pentanucleotide sequence of 5'-TGACT-3'. (Author abstract) (22 Refs)

30. EVIDENCE FOR SIMIAN VIRUS 40 LATE TRANSCRIPTIONAL CONTROL: MIXED INFECTIONS OF WILD-TYPE SIMIAN VIRUS 40 AND A LATE LEADER DELETION MUTANT EXHIBIT TRANS EFFECTS ON LATE VIRAL RNA SYNTHESIS.

Alwine JC
Dept. Microbiology, Sch. Medicine, Univ. Pennsylvania, Philadelphia, PA, 19104
J Virol; 42(3):798-803 1982

Mixed infections involving equal multiplicities of wild-type simian virus 40 and viable deletion mutant dl861 resulted in decreased cytoplasmic levels of wild-type-derived late mRNA, as well as very low to undetectable levels of mutant-derived late mRNA, as compared with individual infections. The dl861 deletion removes 16 to 25 base pairs from the late leader region. This deletion was shown to be the direct cause of the mixed-infection effect; replacement of the deletion with wild-type se-

quences restored normal levels of late mRNAs in mixed infections. Other viral functions, eg, early gene expression and replication, were found to be unaffected by the dl861 deletion. Further examination of the mixed-infection effect showed that the levels of unspliced nuclear precursors of late mRNA, derived from both the mutant and wild-type genomes, were decreased or undetectable, in accord with the cytoplasmic results. Thus, the effect appears to be occurring at the transcriptional level. These data demonstrate a trans-acting effect on late transcription, which is detectable due to the presence of the dl861 mutant in the mixed infection. This finding is indicative of a diffusible factor which exerts a control on simian virus 40 late gene expression at the transcriptional level. A model for positive control of simian virus 40 late gene expression is presented. (Author abstract) (14 Refs)

31. MUTATION AFFECTING LATE GENE EXPRESSION IN POLYOMA VIRUS MAPS IN THE LATE REGION.

Thomas EK, Hare JD
(c/o Hare) Dept. Microbiology, Univ. Rochester Medical Center, Rochester, NY, 14642
J Virol; 42(2):719-724 1982

A mutation in polyoma virus strain 3049 which results in the overproduction of capsid proteins has been mapped to the late region of the genome between the HindIII site at 45.0 map units and the BamHI site at 58.6 map units. This region contains the coding sequence for VP3 and a portion of VP2 but does not include the late promoters or the coding sequence for the late leaders. The possible role of VP2 or VP3 in the regulation of genetic expression in polyoma virus is discussed. (Author abstract) (29 Refs)

32. ENCAPSIDATION AND EXPRESSION OF THE HERPES THYMIDINE KINASE GENE IN POLYOMA VIRUS.

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(c/o Milman) Dept. Biochemistry, Sch. Hygiene and Public Health, Johns Hopkins Univ., Baltimore, MD, 21205
J Virol; 42(2):691-699 1982

A recombinant DNA of 5,150 base pairs was prepared containing the intact early region of polyoma virus, including the viral origin of replication and the structural sequences of the herpes simplex virus Type 1 thymidine kinase gene. Although no thymidine kinase activity was detected when herpes structural sequences alone were transfected into cells, activity was produced when the structural gene followed the polyoma early region. The recombinant DNA was encapsidated into polyoma virions when cotransfected into mouse 3T6 cells with helper DNA from an early polyoma virus mutant. Herpes thymidine kinase activity was detected by a rapid in situ autoradiographic assay in which [125I]iododeoxycytidine was utilized as a substrate for the viral but not the cellular enzyme. (Author abstract) (15 Refs)

33. HERPESVIRUS GLYCOPROTEIN SYNTHESIS AND INSERTION INTO PLASMA MEMBRANES.

Pesko ML, Nystrom P, Pizer LI
(c/o Pizer) Dept. Microbiology and Immunology, Univ. Colorado Health Sciences Center, Denver, CO, 80262
J Virol; 43(2):678-690 1982

In the presence of the antibiotic tunicamycin (TM), glycosylation of herpes simplex virus glycoproteins is inhibited and non-glycosylated polypeptides analogous to the glycoproteins are synthesized. The synthesis of viral proteins and DNA occurs in TM-treated cells. By electron microscopy, nucleocapsids can be observed both in the nucleus and the cytoplasm and TM-treated cells; a small number of enveloped virions were observed on the cell surface. Analyses of the proteins in partially purified virus readily detects viral glycoproteins in the control cells, but neither glycoproteins nor nonglycosylated polypeptide analogs were observed in the virus prepared from TM-treated cells. By labeling the surface of infected cells with 125I, viral glycoproteins were detected as soon as 90 min after infection even when protein synthesis was inhibited with cycloheximide and glycosylation was blocked with TM. Labeling the proteins synthesized in infected cells with [35S]methionine showed that the surface glycoproteins detected in the cycloheximide- and TM-treated cells were not synthesized de novo after infection, but were placed on the cell surface by the infecting virus. Studies with metabolic inhibitors and a temperature-sensitive mutant blocked early in the infectious cycle showed that glycoproteins gA/gB and gD were synthesized soon after infection, but that the synthesis of gC was delayed. Under conditions of infection, in which gC and its precursor pgC are not produced, we have been able to observe the relationships between the glycosylated polypeptides that correspond to pgA/pgB and the nonglycosylated analog made in the presence of TM. (Author abstract) (47 Refs)

34. MECHANISM OF RESISTANCE TO ARA-C IN A MUTANT HERPESVIRUS (MEETING ABSTRACT)

Schnipper LE, Crumpacker CS, Antman K, Kufe D
Harvard Medical School, Boston, MA, 02215
Proc Am Assoc Cancer Res; 23:868 1982

Ara-A (adenine arabinoside) (ara-A) and cytosine arabinoside (ara-C) are effective inhibitors of cellular and Herpes Simplex virus type 1 (HSV-1) DNA synthesis. A mutant of HSV-1 (Ara-Ar) selected in ara-A, and resistant to both ara-A and ara-C has been employed to study mechanisms of resistance to arabinofuranosyl derivatives. Incorporation of ara-C into wild type HSV-1 (KOS) and HSV-1 (ara-Ar) DNA was monitored by isopycnic centrifugation of viral DNA with 3H-ara-C and 32P 6-20 hours post-infection of Vero cells. 32P labelling serves as a measure of relative rates of DNA synthesis. At a concentration of 5 x 10-7M ara-C, the ratio of 3H-ara-C to 32P incorporation into DNA was 0.1 for HSV-1 (ara-Ar) compared to 1.0 for HSV-1 (KOS). When compared to KOS, DNA synthesis with Ara-Ar was less inhibited by ara-C up to a concentration of 5 x 10-6M and was associated with formation of less ara-C DNA. Above 5 x 10-6M there is linear incorporation of 3H-ara-C into mutant viral DNA, and inhibition of DNA synthesis comparable to that in HSV-1 (KOS). High pressure liquid chromatographic analysis of purified viral DNA digested to 3'-nucleotides demonstrates that ara-C is incorporated into internucleotide linkage in both wild type and mutant viruses, between 10(-7)-10-5M. At high concentrations (greater than 10-5M), a greater proportion of ara-C residues are present at the chain terminus. The extent of ara-C incorporation into DNA correlates with inhibition of plaque formation such that HSV-1 (ara-Ar) is insensitive to concentrations less than 5 x 10-6M, whereas HSV-1 (KOS) plaque formation is inhibited in linear fashion between 10(-7)-10-4M. HSV-1 (ara-Ar) resistance to ara-C is overcome above concentrations of 5 x 10-6M. Resistance to ara-C is closely related to exclusion of this analog from viral DNA, however, the precise enzymatic mechanism is not yet understood. (no Refs)

VIRAL GENETICS

35. MAPPING OF AN ADENOVIRUS FUNCTION INVOLVED IN THE INHIBITION OF DNA DEGRADATION.

Lai Fatt RB, Mak S
Dept. Biology, McMaster Univ., Hamilton, Ontario, L8S 4K1, Canada
J Virol; 42(3):969-977 1982

A function involved in the inhibition of DNA degradation has been assigned through complementation tests to a product of region E1b of the adenovirus genome (between 4.5 and 10.5 map units). DNA degradation induced by the adenovirus Type 12 (Ad12) cyt mutant H12cyt70 and the Ad5 early deletion mutant dl313 (with the deletion between 3.5 and 10.7 map units) was inhibited by coinfection with Ad5 region E1a (between 0 and 4.5 map units) mutants dl312 and hr1 and region E1b mutant hr6. The defect of inhibition of DNA degradation in Ad5 dl313 was also complemented in 293 cells. This DNase-inhibitory function does not appear to involve polypeptide IX or the 58,000-dalton polypeptide. Wild-type Ad12 induced DNA degradation in hamster embryo cells, suggesting that the DNase-inhibitory function is not expressed in these nonpermissive cells. Additional evidence suggests the involvement of a second viral product which positively influences the DNase activity and which appears to be an early function. (Author abstract) (47 Refs)

36. CHARACTERIZATION OF A TEMPERATURE-SENSITIVE FIBER MUTANT OF TYPE 5 ADENOVIRUS AND EFFECT OF THE MUTATION ON VIRION ASSEMBLY.

Chee-Sheung CC, Ginsberg HS
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J Virol; 42(3):932-950 1982

A temperature-sensitive, fiber-minus mutant of Type 5 adenovirus, H5ts142, was biochemically and genetically characterized. Genetic studies revealed that H5ts142 was a member of one of the three apparent fiber complementation groups which were detected owing to intracistronic complementation. Recombination analyses showed that it occupied a unique locus at the right end of the adenovirus genetic map. At the nonpermissive temperature, the mutant made stable polypeptides, but they were not glycosylated like wild-type fiber polypeptides. Sedimentation studies of extracts of H5ts142-infected cells cultured and labeled at 39.5 C indicated that a limited number of the fiber polypeptides made at the nonpermissive temperature could assemble into a form having a sedimentation value of 6S, but that this 6S structure was not immunologically reactive. When H5ts142-infected cells were shifted to the permissive temperature, 32 C, fiber polypeptides synthesized at 39.5 C were as capable of being assembled into virions as fibers synthesized in wild type-infected cellsy. In H5ts142-infected cells incubated at 39.5 C, viral proteins accumulated and aggregated into particles having physical characteristics of empty capsids. When the infected culture was shifted to 32 C, DNA appeared to enter the empty particles and complete virions developed. The intermediate particles obtained had the morphology of adenoviruses, but they contained less than unit-length viral genomes as measured by their buoyant density in a CsCl density gradient and the size of their DNA as determined in both neutral and alkaline sucrose gradients. The reduced size of the intermediate particle DNA was demonstrated to be the result of incompletely packaged DNA molecules being fragmented during the preparative procedures. Hybridization of labeled DNA extracted from the intermediate particles to filters containing restriction fragments of the adenovirus genome indicated that the molecular left end of the viral genome preferentially entered these particles. (64 Refs)

IMMUNOLOGICAL STUDIES

37. IMMUNOSURVEILLANCE AND ACOUSTIC 'STRESSING SITUATION' IN THE EXPERIMENTAL SYSTEM BALB/C MOUSE-TSC-3T3 TUMOR.

Favre R, Grallan B, Verrier Ch, Serafino X, Carcassonne Y
Institut J. Paoli et I. Calmettes, 232, boulevard de Sainte-Marguerite, 13273 Marseille Cedex 2, France
C R Acad Sci [III] (Paris); 294(3):159-161 1982

The effect of ultrasound-induced repeated acoustic stress (25,000 Hertz, 30-160 Watts, 5 min/day, on days 2-23 after tumor grafting) on t growth of Tsc-3T3 tumors (4 x 103 to 5 x 105 cells/animal) was studied in 64 BALB/c mice in four groups of 16 animals in each. Two of the four groups were protected by weekly injections of polyoma virus (10(7) plaque-forming units). In the experiments with the lowest tumor cell dose the tumor take rates were 1/16 in the group treated with ultrasound and polyoma virus (Group 1), in the group treated with ultrasound (Group 2), in the group treated with polyoma virus (Group 3) and 0/16 in the group which was not treated after tumor grafting (Group 4). In the experiments with the highest tumor cell dose the tumor take rates were 0/15 in Group 1, 16/16 in Group 2, 2/16 in Group 3 and 16/16 and Group 4. The findings indicate that the ultrasonic stress situation had no effect on the tumor take rates, nor on the protective effect of polyoma virus against the tumor graft. All immunological tests performed were unaltered in the stressed animals compared with the nonstressed ones. (11 Refs) (In FRE)

38. RNA AND PROTEIN SYNTHESIS ARE REQUIRED FOR THE DEVELOPMENT OF INCREASED SENSITIVITY TO NATURAL KILLING IN RAJI CELLS FOLLOWING P3HR-1 VIRUS SUPERINFECTION (MEETING ABSTRACT)

Blazar BA, Fitzgerald J, Strome M
Brigham and Women's Hospital, Harvard Medical School, Boston, MA, 02115
Proc Am Assoc Cancer Res; 23:1057 1982

Sensitivity to natural killing (NK) by normal human peripheral blood lymphocytes develops in normally unsusceptable Raji cells after superinfection with the P3HR-1 strain of Epstein-Barr Virus. This sensitivity to NK is measured as specific 51Cr release in 4 hr assays at 100:1 effector target ratios. NK sensitivity is due to an early membrane change that does not require DNA synthesis since this sensitivity develops in superinfected Raji cells cultured in the presence of cytosine arabinoside (Ara-c) (10 ug/ml). NK sensitivity does not develop in P3HR-1 superinfected Raji cells that are cultured in the presence of the RNA synthesis inhibitor actinomycin D (1 ug/ml) and drops to half the amount usually detected in superinfected cells that are grown with the protein synthesis inhibitor cyclohexamide (50 ug/ml). Removal of the 24 hr cyclohexamide block restores the sensitivity to NK to that of the untreated P3HR-1 infected cells. These findings suggest that the development of NK sensitivity in Raji cells following superinfection is due to the addition to the cell membrane of a virally promoted

molecule (s) that requires active RNA and protein synthesis. (no Refs)

39. AN ADOPTIVE CELL TRANSFER SYSTEM FOR THE EVALUATION OF IMMUNITY TO HERPES SIMPLEX VIRUS IN MICE.

Parrila L, Howes EL, Taylor WA, Mitchison NA
(c/o Mitchison) Tumor Immunology Unit, Zoology Dept., Imperial Cancer Res. Fund, Univ. Coll. London, Gower St., London WC1E 6BT, England
Clin Exp Immunol; 48(2):307-314 1982

Lymphocytes adoptively transferred from syngeneic immune donors protect mice against challenge with Herpes simplex virus Type 1. Normal mice require transfer of 3 x 10(7) spleen cells for protection. Sublethal irradiation (450 rad) decreases the number required five-fold. Lymphocytes from non-immune donors do not protect, and hyperimmunization does not enhance the protection efficiency of donors. The viral LD50 varies through a range 10(5)-fold during the period of recovery from this amount of radiation, but over the same period there is little variation in the number of cells required for protection. Nor is there much variation in this number between strains of mice naturally susceptible (CBA) and resistant (C57) to this virus. We conclude that natural resistance operates at a level of virus handling prior to operation of the lymphocyte system, perhaps at the generation of interferon. As few as 1.3 x 10(6) immune T lymphocytes can protect against challenge provided that they are transferred together with normal spleen cells. We conclude that primed lymphocytes act in cooperation with non-immune cells. (Author abstract) (18 Refs)

OTHER RELATED STUDIES

40. EXPRESSION OF TRANSDUCED GENES IN MAMMALIAN CELLS (MEETING ABSTRACT)

Berg P
Department of Biochemistry, Stanford University Medical Center, Stanford, CA
Thirty-fifth Annual Symposium on Fundamental Cancer Research, March 2-5, 1982, Houston, Texas. The University of Texas M. D. Anderson Hospital and Tumor Institute 38 pp., 1982.

Our laboratory has explored two approaches to achieving transduction-transformation of mammalian cells. One uses recombinants constructed in vitro from suitably modified cloned cDNAs or prokaryote and eukaryote-derived genomic segments and SV40 DNA from which segments encoding early and late viral functions have been excised. In this experimental design, the recombinant viral genomes must contain the origin of SV40 DNA replication and must not be larger than 5.3 kb in order to be propagated as mature virion genomes; moreover, since the recombinant genomes are defective, propagation can occur only with a helper virus to provide the missing gene product(s). A virtue of this approach is that amplification of the recombinant genomes ensures a high yield of the mRNAs and proteins expressed from the transduced genes. The second approach exploits a new group of transducing-transforming DNA vectors for the introduction and maintenance of new genes in a variety of mammalian cells. One prototype of this class of vectors, pSV2-neo, developed by Peter Southern and Bruce Howard, contains a DNA segment from the plasmid pBR322, in order to permit propagation of the recombinants in Escherichia coli, and a transcription unit comprising the SV40 early region promoter, the dominant selectable marker, Econeo, an intervening sequence, and a poly A site. Cells transformed with pSV2-neo (frequency, 10(-4) to 10(-5)) grow in media containing the inhibiting aminoglycoside G418. Unique restriction sites in pSV2-neo DNA permit the introduction of other, nonselectable genes into the same vector and, thereby, the cotransformation of cells for these otherwise nonselectable phenotypes. (no Refs)

41. TUMOR VIRUSES AND EARLY MOUSE EMBRYOS.

Kelly F, Condamine H
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Biochim Biophys Acta; 651(2/3):105-141 1982

Experiments of infection of early mouse embryos with DNA and RNA tumor viruses are reviewed and expression of various types of endogenous RNA viruses is addressed. Viruses discussed include simian virus 40, polyoma virus, Moloney murine leukemia virus, and Type A and C endogenous viruses. Studies performed with embryonal carcinoma cells and simian virus 40, polyoma viruses, and murine RNA tumor viruses are reviewed. (247 Refs)

42. SUSCEPTIBILITY OF BALB/CGNDU MICE TO TRANSPLANTABLE TUMORS, IN VITRO TRANSFORMED CELLS, BK AND SV40 VIRUSES, AND CHEMICAL CARCINOGENS.

Morrison DG, Moyer MP, Lynd FT, Rogers W, Moyer RC
Thorman Cancer Res. Lab., Trinity Univ., 715 Stadium Drive, San Antonio, TX, 78284
Oncology; 39(4):228-233 1982

Various oncogenic agents were employed in tumor susceptibility studies of an inbred subline of BALB/cGn mice, BALB/cGnDu, which carries a mutant gene that results in spontaneous thymic involution in homozygous recessive individuals. Of 18 transplantable tumors, 3 in vitro transformed cell lines, 2 oncogenic papovaviruses and 3 chemical carcinogens evaluated, only the Harding-Passey amelanotic melanoma produced tumors in 100% of the normal adult mice injected. The S180 pleomorphic sarcoma produced tumors in 56% of normal adult mice. Although not tumorigenic in adults, the mKSA, Morris (rat) hepatoma Number 7777, and a human papovavirus-transformed human cell induced mouse tumor line [GI110(BK) B6D2 Tu] formed tumors in 100, 62 and 70%, respectively, of recipients inoculated as neonates. SV40 and human papovavirus BK virions, as well as SV40, BK, and spontaneous in vitro transformed BALB/cGnDu cells were not tumorigenic. Although the histocompatibility antigens of these animals have not been studied, no correlations could be made between the tumor susceptibility of these animals with regard to the commonly employed inbred line BALB/c nor with regard to the range of host susceptibility of the transplantable tumors. This inbred subline represents a new strain of mice whose genetic defect makes it useful for transplantation immunology and physiological genetics. (Author abstract) (13 Refs)

43. COMPLEMENTATION FOR REPLICATIVE FORM DNA REPLICATION OF A DELETION MUTANT OF H-1 BY VARIOUS PARVOVIRUSES.

Rhode SL
Inst. Medical Res. Bennington, VT, 05201
J Virol; 42(3):1118-1122 1982

A defective interfering genome of the parvovirus H-1 called dl1, has been isolated and characterized. The only alteration in

dl1 that has been detected is a 300-base-pair deletion at map position 38. Replicative form DNA replication of dl1 requires the RF rep function of a helper virus and the parvoviruses H-1, H-3, MVM, or MVM(i), but not LuIII complement dl1 for replicative form DNA replication. (Author abstract) (8 Refs)

44. RABBIT TESTICLE CELL PLAQUE TECHNIQUE FOR HERPESVIRUS HOMINIS TYPE 1 AND TYPE 2.

Thiel KD, Wutzler P, Schweizer H
Institut fur Medizinische Mikrobiologie der Medizinischen, Medizinische Akademie Erfurt, DDR-506 Erfurt, E. Germany
Acta Microbiol Acad Sci Hung; 29(1):67-68 1982

Monolayer cultures of rabbit testicle cells were found to be highly suitable for plaque technique with Herpes virus hominis Type 1 and Type 2. The advantages of these cells for plaque technique using an overlay of methylcellulose are described. Plaque formation was found to be rapid and abundant compared with other cell lines. (3 Refs) (In GER)

45. MONOCLONAL ANTIBODIES FROM MICE BEARING POLYOMA VIRUS-INDUCED TUMOR.

Moav N, Smorodinsky NI, Balin BA, Witz IP
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Cancer Immunol Immunother; 12(3):217-224 1982

Antibodies directed against antigens expressed on SEYF-a cells, a polyoma virus-induced sarcoma propagated in syngeneic A.BY mice, were studied and the binding characteristics of some of the resulting autologous monoclonal antibodies to various tumor cell lines and other properties of these antibodies are described. Spleen cells from SEYF-a-bearing mice 4-6 wk after transplantation were used for the hybridization experiments; 45 hybridomas were found to be positive. The antibodies produced by hybridoma F4/5.10 demonstrated high binding activity only to SEYF-a cells and very low binding to KREBS and ELD cells. Results suggested that F4/5.10 produces antibody directed against an antigenic determinant present predominantly on the SEYF-a cell membrane. Three of hybrid clones were found to produce IgG2a, three produced IgG1, and one produced IgM. The heavy chains of F4/5.10 and P3 were in the gamma-chain region, whereas the heavy chains of F7/129.7 were in the mu-chain region. When antibodies from F4/5.10 were used only SEYF-a cells (and none of the other tumor cells tested) absorbed considerable amounts of the antibodies (65%). These results, together with the results obtained from the binding experiments, strongly support the assumption that F7/129.7 antibodies are directed against a broadly distributed public determinant, while F4/5.10 antibodies are directed against a specificity that has a narrow distribution. (33 Refs)

46. ANTIVIRAL EFFECTS OF INTERFERON ON A SOMATIC CELL HYBRID BETWEEN TWO BURKITT'S LYMPHOMA CELL LINES OF DIFFERENT INTERFERON SENSITIVITIES.

Lidin B, Lamon EW
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Infect Immun; 36(2):847-849 1982

A somatic cell hybrid between two human Burkitt's lymphoma cell lines, Raji and Daudi, was infected with either Epstein-Barr virus or vesicular stomatitis virus after interferon treatment. Raji cells are resistant to the antiviral effects of exogenously added interferon, whereas Daudi cells are interferon sensitive. The Raji-Daudi hybrid showed an interferon sensitivity that was intermediary to that of the parental cells against both viruses. (Author abstract) (14 Refs)

47. DNA-MEDIATED GENE TRANSFER: RECOMBINATION BETWEEN COTRANSFERRED DNA SEQUENCES AND RECOVERY OF RECOMBINANTS IN A PLASMID.

Anderson RA, Krakauer T, Camerini-Otero RD
Genetics and Biochemistry Branch, Natl. Inst. Arthritis, Diabetes, Digestive and Kidney Diseases, NIH, Bethesda, MD, 20205
Proc Natl Acad Sci USA; 79(9):2748-2752 1982

Ltk- aprt- mouse L cells were transformed to the tk+ phenotype with 10 ng of the herpes simplex virus-1 thymidine kinase (tk) gene and 20 ug of pBR322 or simian virus 40 (SV40) DNA. DNAs from five cloned cell lines show restriction endonuclease fragments that hybridize to both tk and pBR322 or SV40 DNA. In all of the cell lines some of these fragments also contain cellular DNA sequences. The use of carrier DNAs with defined sequences has enabled us to demonstrate that the joining of carrier and selectable gene sequences occurs in mouse cells. In one case we have been able to use the ampicillin resistance marker of pBR322 to 'rescue' a recombinant plasmid. An analysis of the junction between pBR322 and tk in this plasmid suggests that a small area of homology (16 of 19 base pairs) might be involved in the recombination process. (Author abstract) (35 Refs)

48. MONOCLONAL ANTIBODIES AGAINST THE T-CELL CHRONIC LYMPHOCYTIC LEUKEMIA-DERIVED CELL LINE, SKW3 (MEETING ABSTRACT)

Dowell BL, Gore I, McCormick GM, Metzgar RS
Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC, 27710
Fed Proc; 41(3):270 1982

Splenocytes from BALB/C mice immunized with the human T-CLL cell line, SKW3, were fused with the P3 X63 Ag8 myeloma cell lines. Five cloned hybridomas were isolated which were cytotoxic for SKW3, but not for the common acute lymphoblastic leukemia-derived (cALL) cell lines, REH and 697. DU-SKW3-1 is an IgG2b antibody which detect a 65-68,000 dalton antigen present on all peripheral blood T lymphocytes and thymocytes, but absent from normal B lymphocytes, monocytes, granulocytes, and platelets. The antigen was also detectable on T-cell derived leukemias and lymphomas and on B-cell derived CLL cells, but not on myeloid leukemias or on common ALL cells. The epitope detected by DU-SKW3-1 is different from that detected by L17F12. DU-SKW3-2 reacts with a trypsin-sensitive antigen which is preferentially expressed on mature T and B lymphocytes. It was cytotoxic for EBV (Epstein-Barr virus)-transformed B cell lines, myeloma cell lines and HuT78, a Sezary-derived cell line. DU-SKW3-2 was not cytotoxic for four T-ALL derived cell lines or for four cALL-derived cell lines. The monoclonals, DU-SKW3-3,4, and 5 were cytotoxic for normal T cells, B cells and monocytes, but did not react with cALL cell lines. (no Refs)

49. ETIOLOGY OF MOUTH AND LIP TUMORS.

Zabezhinskii MA, Godorozha PD
N. N. Petrov Res. Inst. Oncology, Leningrad, USSR
Vopr Onkol; 28(2):108-113 1982

Literature data on the etiology of mouth and lip cancers are reviewed. Pathogenesis of mouth neoplasms is associated with the exposure to various chemical, physical, and biological carcinogenic factors alone or in combination with the modifying factors. The increased incidence of mouth cancer can be caused by smoking, tobacco chewing, exposure to polycyclic aromatic hydrocarbons, alcohol consumption, and exposure to pesticides. Physical carcinogens include UV, x rays, and sunlight. Experimental data indicate that herpes virus and polyoma virus can induce adenomas and adenocarcinomas of mouth mucosa. Poor hygiene of the mouth can increase the risk of development of mouth cancer. Chronic irritation of the mouth mucosa was shown to enhance penetration of carcinogens into mucosa and to stimulate cell proliferation. Bacterial flora of the oral cavity was shown to contribute to the metabolism of chemical carcinogens. Deficiency of vitamins A and B led to development of precancerous conditions of the mouth mucosa. (74 Refs) (In RUS)

50. PATHOBIOLOGY OF HEPATITIS B VIRUS.

Gerber MA, Thung SN
Dept. Pathology, Mount Sinai Hosp. and Sch. Medicine, City Univ. New York, New York, NY, 10029
Pathobiol Annu; 11:197-228 1981

Knowledge of the pathology of hepatitis B virus (HBV) and more recent findings are discussed. The amount of virus specified unique gene products may be limited, although combined mol wt of all polypeptides from hepatitis B surface antigens (HBsAg), hepatitis B core antigen, and a cryptic form of hepatitis B antigen (HBeAg) appear to exceed the coding capacity of the small HBV genome, which is approx 100,000 daltons of protein. Recent studies indicate that HBsAg and albumin receptor activity emerge simultaneously in hepatocellular cytoplasm of HBV infected patients and also in the cytoplasm, on the cell surface, and in the supernatant culture media of an HBsAg producing hepatocellular carcinoma (HCC) cell line. The 28 nanometer core particles of HBV can be isolated from circulating Dane particles (DP) by detergent treatment or from hepatocellular nuclei of infected liver. Observation of two populations of DP may be of great biological importance since only full particles with complete genome may be infectious, whereas empty particles may be defective and interfere with replication of homologous virus. HBeAg probably is an integral structural component of the HBV nucleocapsid, coded by the genome of the virus. In serum of HBV infected patients, HBeAg occurs free or in association with IgG, most likely as immune complexes. Diseases associated with HBV infection are acute and chronic hepatitis, and a variety of clinical syndromes may be associated with HBV infection. HBV may be one of the agents involved in the etiology of human HCC. HCC in man is highly correlated with prevalence of HBsAg carriers. Serologic markers of HBV infection are found in significantly higher frequency in patients with HCC, and tumor markers are detected in HCC cells. The possibility cannot be excluded that the virus plays a merely passive role in hepatocarcinogenesis. (228 Refs)

51. EXPERIMENTAL TRANSPLACENTAL INFECTION OF PREGNANT DOGS WITH CANINE HERPESVIRUS.

Hashimoto A, Hirai K, Yamaguchi T, Fujimoto Y
Dept. Veterinary Microbiology, Veterinary Hosp., Gifu Univ., Kakamigahara-shi, Gifu 504, Japan
Am J Vet Res; 43(5):844-850 1982

Fetal infection was established in 28 of 33 pups obtained from seven pregnant dogs (47th to 53rd day of gestation) inoculated iv with canine herpesvirus (CHV). The pathogenesis of transplacental CHV infection was investigated by using pathologic examination, immunofluorescence microscopy, and viral assay. Infection with the virus was recognized in 13 of 18 neonatal pups obtained from four bitches by spontaneous delivery at full term. Eleven pups died with a wk of birth and all had characteristic lesions of CHV infection. Thirteen live-born and 2 stillborn pups derived from three bitches by cesarean section died within 9 days of birth. Characteristic lesions of CHV infection were observed in various organs, such as liver, spleen, kidney, and lung. Fourteen of the fetal placentas had variable degree of necrotizing lesions. The lesions were characterized by degenerative changes and focal necrosis in the placental labyrinth. Lesions were present in the walls of allantoic blood vessels. Intranuclear inclusions were evident in degenerating trophoblast cells and in the cells of maternal and fetal blood vessels. Infective virus and viral antigen were detected in the various organs of pups and in the trophoblastic cells of junctional zone. Virus also was found in the walls of maternal and fetal blood vessels of the placental tissues. The results strongly indicated that transplacental transmission of CHV may occur in pregnant dogs at a late stage of gestation. (Author abstract) (20 Refs)

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DNA TUMOR VIRUSES IN NON-PRIMATE SYSTEMS

SERIES CB11 No. 82/11

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VIRUS ISOLATION/ STRUCTURE/ CHARACTERIZATION/ IDENTIFICATION

1. IMPROVED INFECTIVITY OF REASSEMBLED POLYOMA VIRUS.

Yuen LK, Consigli RA
Div. Biology, Section Virology and Oncology, Kansas State Univ., Manhattan, KS, 66506
J Virol; 43(1):337-341 1982

Polyoma virus was dissociated into capsomeres (18, 12, and 5S) and a DNA-protein complex (48S) with Ca++ chelator, ethyleneglycol-bis- ethyleneglycol-bis-N,N'-tetraacetic acid, and the reducing agent, 2-mercaptoethanol. The reaction was maintained at pH 5.0. Reassembly of the dissociated components to complete virions was accomplished by dialyzing these components overnight at 4 C against the reassembly buffer containing CaCl2, dimethylsulfoxide, Triton X-100, and 0.01 M Tris-acetic acid (pH 5.0). Reconstituted particles ranged from 240S complete virions to lighter intermediate species. Approximately 25% of the dissociated particles could be physically reassembled to complete virions. These virions regained 12.5% of their hemagglutination ability and as much as 6.7% of their

HE 20.3173/2:

original infectivity. The infectivity of these reassembled particles represented a 100-fold increase in infectivity compared with that of the particles that were dissociated and reassembled at pH 7.4. Biochemical analysis showed that the polyoma viral receptor of the virions reassembled at pH 7.4 was greatly reduced, whereas virions reassembled at pH 5.0 retained their receptor. Reassembly could be further improved by additions of either exogenous capsomeres or DNA-protein complex to the reassembly reaction mixture. (Author abstract) (18 Refs)

2. THE COMPLETE NUCLEOTIDE SEQUENCE OF POLYOMA VIRUS AND IDENTIFICATION OF THE TRANSFORMING (CARCINOGENIC) GENES.

Soeda E
Natl. Inst. Genetics, Mishima, Shizuoka Prefecture 411, Japan
Nippon Nogeikagaku Kaishi; 56(1):31-37 1982

The polyoma virus is a small-type tumorigenic virus composed of a circular double strand DNA of 5,292 bases. The complete nucleotide sequence of the polyoma virus is described, and the mechanism of carcinogenesis of cells by the virus is clarified. Genetic analysis of the mutated region of wild and non-transformed strains of N18 is performed, and differences between the patterns of carcinogenesis of other papovaviruses (SV40, BKV, etc) and that of the polyoma virus are discussed. (39 Refs) (In JPN)

3. POLYOMA-LIKE PARTICLE: CHARACTERIZATION OF THE DNA ENCAPSIDATED IN VITRO BY POLYOMA EMPTY CAPSIDS.

Sillaty SN, Berns KL, Aposhian HV
Dept. Cellular and Developmental Biology, Univ. Arizona, Tucson, AZ, 85721
J Biol Chem; 257(11):6571-6575 1982

Polyoma empty capsids and DNA interact in a cell-free system to form a polyoma-like particle (PLP). The particle is stable in high concentrations of salt and contains a DNA fragment that is protected by the capsid from the action of DNase I. The DNA extracted from PLP is heterogeneous in size. It has a mean mol wt of 1.2 x 10(6) with a standard deviation of +-0.5 x 10(6). Analysis of PLP DNA with restriction endonucleases and the Southern blot technique reveals that a specific primary sequence is not required for PLP formation. Either linear, circular, or supercoiled polyoma DNA, as well as single-stranded DNA, rRNA, and the synthetic homopolymers poly(dA)-poly(dT) and poly(dG)-poly(dC) can be used for PLP formation. It is concluded that polyoma empty capsids can be used to bind and encapsidate nucleic acids differing in their primary, secondary, and superhelical tertiary structure to form, in vitro, stable polyoma-like particles. (Author abstract) (28 Refs)

4. HERPESVIRUS SAIMIRI STRAIN VARIABILITY.

Desrosiers RC, Falk LA
Harvard Medical Sch., New England Regional Primate Res. Center, Southborough, MA, 01772
J Virol; 43(1):352-356 1982

Herpesvirus saimiri was isolated from 22 squirrel monkeys by cocultivation of peripheral lymphocytes with permissive owl monkey kidney monolayer cells. Comparison of virion DNA fragments produced from restriction endonuclease digestion was used as a sensitive measure of strain variability. Although all isolates contained similarities and common features, 19 of 22 were readily distinguished. Three of the isolates, however, were indistinguishable and possibly were related epidemiologically. Distinct subtypes of H saimiri were not evident by these criteria; Peruvian, Colombian, Guyanan, and Bolivian squirrel monkeys yielded isolates with characteristic features peculiar to the geographic region. Three of three colony-born squirrel monkeys that were tested yielded a strain of virus distinct from that obtained from the mother. In separate experiments, two of three animals chosen at random yielded a strain of virus different from that originally obtained 16 and 22 months previously; only one of the three animals examined yielded the same strain of virus 22 months after the original isolation. The degree of restriction endonuclease fragment variability among H saimiri strains appeared to be greater than previously observed for other herpesviruses. (Author abstract) (23 Refs)

5. ORGANIZATION OF THE EPSTEIN-BARR VIRUS DNA MOLECULE. II. FINE MAPPING OF THE BOUNDARIES OF THE INTERNAL REPEAT CLUSTER OF B95-8 AND IDENTIFICATION OF ADDITIONAL SMALL TANDEM REPEATS ADJACENT TO THE HR-1 DELETION.

Hayward SD, Lazarowitz SG, Hayward GS
Dept. Pharmacology and Experimental Therapeutics, Johns Hopkins Univ. Sch. Medicine, Baltimore, MD, 21205
J Virol; 43(1):201-212 1982

We used cloned BamHI fragments from Epstein-Bar virus strain B95-8 [EBV(B95-8)] DNA to obtain detailed restriction maps of the region of the genome adjacent to the large internal repeat cluster. These maps together with the results of hybridization experiments using a 3.1-kilobase repeat probe defined more precisely the location of the junctions between the internal repeat cluster and the flanking unique-sequence DNA. On one side (UL), the repeat sequences extended 600 +-80 base pairs (bp) into BamHI-Y; on the other side (Us), they extended 1,300 +-200 bp into BamHI-C. Therefore, EBV(B95-8) DNA contained a nonintegral number of 3.1-kilobase repeat units, namely, 12.6 copies. The mapping studies also revealed a second series of internal tandem repetitions in EBV(B95-8) DNA located within the BamHI-H fragment. This cluster comprised 11 copies of a 135-bp repeat unit which contained a single site for the NotI restriction endonuclease. Hybridization to these cloned EBV(B95-8) fragments using total EBV(HR-1) DNA as probe indicated that the deletion in EBV(HR-1) removed all 3,000 bp of unique-sequence DNa which lay between the large 3.1-kilobase and the small 135-bp repeat clusters. Thus, the deletion which destroyed the transforming ability in the EBV(HR-1) virus was bounded on either side by tandem repetitions. (Author abstract) (25 Refs)

VIRUS ASSOCIATED CELL TRANSFORMATION AND HOST ALTERATION

6. SIMIAN VIRUS 40 A GENE FUNCTION: FURTHER CHARACTERIZATION AND GROWTH OF TSA TRANSFORMED CHINESE HAMSTER CELLS.

Robinson CC, Lehman JM
(c/o Lehman) Dept. Pathology, Univ. Colorado Sch. Medicine, Denver, CO, 80262
J Cell Physiol; 111(3):225-231 1982

Characterization of the tsA transformants and their growth pattern on normal cell monolayers were studied using two simian virus 40 (SV40) transformed Chinese hamster embryo

cell lines, ChHA58, transformed by group A mutant ts58, and the WT virus transformed cell line, ChHWT. Characteristics used to evaluate transformation of these cells are summarized. tsA mutant transformed ChH embryo cells at 40.5 C were distributed in all phases of the cell cycle and had heavily labeled nuclei after a short 3H-thymidine label indicating that they continue DNA synthesis at the restrictive temperature for T antigen. tsA transformed cells were seeded onto monolayers of normal Chinese hamster cells. Colonies of tsA transformed cells appeared at 37 C but not at 40.5 C while no difference in colony forming ability of WT transformants was observed at these two temperatures. During these experiments no debris accumulated in the culture fluid at 40.5 C, unlike the large numbers of floating dead cells present when the tsA transformants were grown alone. Results suggested that the growing pattern of tsA mutant transformed cells at 40.5 C might be different under the two culture conditions. Viable cells were necessary to obtain growth arrest since this effect was not observed with glutaraldehyde fixed cells or conditioned medium. These experiments demonstrated that under proper substrate conditions, tsA Chinese hamster cells were arrested in DNA synthesis. (26 Refs)

7. INCREASED EXPRESSION OF SV40 T ANTIGEN AND CELL DIVISION IN SKIN FIBROBLAST CELL LINES DERIVED FROM A FAMILY AT HIGH RISK OF CARCINOMA (FAMILY G OF WARTHIN).

Lubiniecki AS, Lynch HT, Blattner WA, Guirgis H
Flow Lab., Inc., Old Springhouse Road, McLean, VA, 22102
J Cancer Res Clin Oncol; 103(2):127-133 1982

T-antigen expression in 24 of 31 skin fibroblast cell lines from members of Family G was found to be significantly elevated compared with a healthy control population. However, the pattern of elevation did not appear to be associated with cancer risk. In addition, T-antigen vhlues were independent of the age and sex, as well as the branch of family and generation of the cell donor. Cell lines from Family G tended to divide more frequently than cell lines from control donors. This tendency was negatively associated with elevated T-antigen expression in Family-G cell lines, while control cell lines showed no such correlation. These results demonstrate that caution must be taken in evaluating potential markers of cancer risk. Superficial analysis of the data would indicate the utility of the T-antigen assay as a marker for cancer family syndrome. However, consideration of additional factors, including pedigree relationships and cell biology in vitro, indicate that SV40 T-antigen expression is an ambiguous marker of this type of cancer risk, even when large groups are studied. (Author abstract) (14 Refs)

8. HYBRIDS FROM FUSION OF TUMOR CELLS WITH NORMAL AND SV40 TRANSFORMED HUMAN FIBROBLASTS HAVE A FINITE IN VITRO LIFESPAN (MEETING ABSTRACT).

Pereirasmith OM, Smith JR
W. Alton Jones Cell Science Center, Inc., Old Barn Road, Lake Placid, NY, 12946
In Vitro; 18(3,part2):283 1982

Human diploid cells have finite division potential in vitro, while tumor derived and virally transformed human cells divide indefinitely. To investigate the processes leading to the acquistion of indefinite proliferation, we have studied the proliferative potential of hybrids from normal x tumor and tumor x SV40 transformed cell fusions. We have previously found that 30-50% of hybrids from normal x SV40 transformed cell fusions were capable of extensive division (greater than 10 PD). However, when normal fibroblasts were fused with various human tumor cell lines, only 3% or less of the hybrids were able to divide extensively. It is therefore possible that different processes lead to indefinite proliferation in the SV40 vs tumor derived cell lines. If this is the case, tumor x SV40 transformed cell fusions might yield hybrids with finite lifespan. Fusion of an SV40 transformed human fibroblast with various tumor cell lines yielded 20% or fewer hybrids capable of extensive proliferation. When the tumor line was derived from a carcinoma, glioma or osteosarcoma, these hybrids eventually stopped dividing. Fusion with a fibrosarcoma, however, yielded hybrids that achieved at least 100 PD. These results demonstrate the dominance of the finite lifespan phenotype in normal x immortal cell fusions and suggest that different indefinitely proliferating lines arise due to loss of different specific regulatory functions. (no Refs)

9. FUSION-INDUCED DIFFERENTIATION OF SV40-INFECTED HUMAN KERATINOCYTES.

Steinberg ML, Defendi V
Dept. Pathology, New York Univ. Medical Center, New York, NY, 10016
Exp Cell Res; 139(2):369-375 1982

Cells in cultures of human keratinocytes infected by the oncogenic virus, SV40, were fused by exposure to polyethylene glycol (PEG). Fusion induced differentiation of the infected cells within 1-2 days, as measured by staining with orange G/ acid fuchsin and by the production of cornified envelopes. The extent of induced differentiation was found to be correlated with the degree of multinucleation. The inductive effect of fusion was manifest during the early serial passages post-infection, but was eventually lost as the keratinocytes became increasingly transformed over time. (Author abstract) (18 Refs)

10. CHARACTERIZATION OF AN AMINO ACID FUCOSIDE OF NORMAL AND SV40-TRANSFORMED HUMAN EMBRYONIC LUNG CELLS.

Morton PA, Klinger MM, Steiner SM
(c/o Steiner) T. H. Morgan Sch. Biological Sciences, Univ. Kentucky, Lexington, KY, 40506
Cancer Res; 42(8):3022-3027 1982

The incorporation of radioisotopically labeled fucose into a prominent fucosylated component, ie, fucose-labeled amino acid fucoside 4c (FL4c), of human embryonic lung cells is markedly decreased in cell lines derived from human tumors. In the current study, we have extended the above observations by examining more closely related normal and transformed human cells, eg, W138 cells and SV40-transformed W138 cells. We have found that the level of radioisotopically labeled fucose incorporated into FL4c of the SV40-transformed human embryonic lung cells is dramatically reduced as compared to their normal counterpart cells. Additionally, we have chemically characterized FL4c. FL4c was purified from monkey tissues using a combination of gel filtration chromatography, ion-exchange chromatography, and preparative thin-layer chromatography. Analysis of this material was accomplished by amino acid analyzer and by gas-liquid chromatography; fucose, glucosamine, and aspartic acid in molar ratios of approximately 1.0:1.0:1.0 were observed. Furthermore, alpha-L-fucosidase treatment of [3H]fucose-labeled FL4c revealed that the fucose was alpha-linked and in a terminal position. Similar treatment of [3H]glucosamine-labeled FL4c yielded a component that cochromatographed with N-acetylglucosaminylasparagine. The

combined results of the structural studies are consistent with the structure of FL4c being alpha-fucosyl-N-acetylglucosaminylasparagine. (Author abstract) (25 Refs)

11. CARBOXY TERMINUS OF POLYOMA MIDDLE-SIZED TUMOR ANTIGEN IS REQUIRED FOR ATTACHMENT TO MEMBRANES, ASSOCIATED PROTEIN KINASE ACTIVITIES, AND CELL TRANSFORMATION.

Carmichael GG, Schaffhausen BS, Dorsky DI, Oliver DB, Benjamin TL
Dept. Microbiology, Univ. Connecticut, Sch. Medicine, Farmington, CT, 06032
Proc Natl Acad Sci USA; 79(11):3579-3583 1982

We have constructed a transformation-defective polyoma virus mutant (Py 1387-T) that directs the synthesis of a normal small tumor antigen, a functional large tumor antigen, and a truncated (51,000-dalton) middle-sized tumor (mT) antigen that lacks 37 amino acids at its COOH terminus. The shortened mT polypeptide is missing the hydrophobic 'tail' thought to be responsible for the anchorage of this protein into the plasma membrane and is in fact in cytosol fractions. This truncated mT polypeptide is inactive in an in vitro protein kinase assay and is altered in its phosphorylation in vivo. Mutant 1387-T differs from wild-type virus in having a T-A base pair instead of a C-G base pair at nucleotide position 1387. This change was introduced into viral DNA by using a synthetic undecanucleotide as a specific mutagen. Wild-type polyoma DNA was rendered single stranded by molecular cloning into coliphage M13. The oligonucleotide, which hybridizes with a mismatch at the site to be altered, was used to prime the synthesis of double-stranded closed circular DNA. Progeny recombinant phages were screened by DNA sequence analysis for the desired base change. The polyoma mutant was reconstructed from recombinant phage replicative form DNA molecules containing the mutation. (Author abstract) (45 Refs)

12. SEQUENCE ORGANIZATION OF A VIRAL DNA INSERTION PRESENT IN THE ADENOVIRUS-TYPE-5-TRANSFORMED HAMSTER LINE BKH268-C31.

Westin G, Visser L, Zabielski J, Van Mansfeld AD, Petterson U, Rozijn TH
Dept. Microbiology, Biomedical Centre, Box 581, S-751 23 Uppsala, Sweden
Gene; 17(3):263-270 1982

The smaller viral insert present in the 268-C31 cell line was cloned using the lambda phage Charon 4A as a vector and the nucleotide sequence of the host-viral junctions. The organization of the flanking eukaryotic sequences was studied. Twenty clones out of 100,000 scored positive in the hybridization tests using intact Ad5 DNA as a probe. Results indicated the presence of many more viral insertions in the BHK268-C31 DNA than the two that could be detected by the conventional reassociation kinetic studies and blotting experiments. (26 Refs)

13. VIRUS-SPECIFIC PROTEINS IN ADENOVIRUS TYPE 12-TRANSFORMED AND TUMOUR CELLS AS DETECTED BY IMMUNOPRECIPITATION.

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Inst. Genetics, Univ. Cologne, 5 Cologne 41, Weyertal 121, W. Germany
J Gen Virol; 59(part2):357-366 1982

Adenovirus Type 12 (Ad12)-specific proteins were determined in nine well-characterized AD12-transformed hamster cell lines and in Ad12-induced hamster and rat tumor lines by immunoprecipitation, gel electrophoresis and autoradiography. All cell lines expressed a 60K mol wt polypeptide. In several cell lines, the presence of a 58K mol wt protein could also be demonstrated by these techniques. Smaller Ad12-specific proteins could be detected only in the Ad12-transformed hamster cell lines HA12/7 and A2497-3, and in the Ad12-induced hamster tumor line CLAC3. The quality of the immunoprecipitation tests performed depended primarily on the sera used; their antibody titers varied widely. For at least one of the Ad12-transformed hamster cell lines (HA12/7), there was good agreement in the number and mol wt of Ad12-specific proteins detected by both immunoprecipitation and in vitro translation of hybrid-selected cytoplasmic RNA. There was no clear-cut correlation between the number and the nature of Ad12-specific proteins and the way in which the cell lines or tumors were obtained. Cell lines with the least number of copies of Ad12 DNA persisting appeared to express the largest number of Ad12-specific proteins. (Author abstract) (34 Refs)

14. EFFECT OF SELENIUM ON THE INDUCTION OF BREAST FIBROADENOMAS BY ADENOVIRUS TYPE 9 AND 1,2-DIMETHYLHYDRAZINE-INDUCED BOWEL CARCINOGENESIS IN RATS.

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Wallenberg Lab., Univ. Lund, Lund, Sweden
Int J Cancer; 29(6):707-710 1982

Selenium in its organic and inorganic forms has been shown to inhibit the development of chemically induced, spontaneous and transplanted tumors. The present investigation was performed to study the effect of selenium (4 ug per ml of drinking water) on tumorigenesis of adenovirus-type-9-induced breast fibroadenomas and on 1,2-dimethylhydrazine-induced bowel carcinogenesis in WF rats. It was found that identical treatment with Se under identical conditions and with no obvious toxic effects on the rats (1) resulted in inhibition of DMH-induced large-bowel carcinogenesis; (2) facilitated induction of small-bowel cancer by the same carcinogen in the same animals, and (3) greatly facilitated induction of breast fibroadenoma by adenovirus Type 9 in the same strain of rats. The effect of Se treatment on DMH-induced large-bowel carcinogenesis confirms previous findings and proves that the opposite effect on fibroadenoma development is not due to differences in eg effective dose, animal strains or condition of the animals. It is not yet clear through which mechanisms Se exerts these effects. (Author abstract) (29 Refs)

15. ANALYSIS OF TYPE 5 ADENOVIRUS TRANSFORMATION WITH A CLONED RAT EMBRYO CELL LINE (CREF).

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Proc Natl Acad Sci USA; 79(11):3527-3531 1982

A recently isolated cloned cell line of Fischer rat embryo fibroblasts (CREF) can be transformed at high frequency by adenovirus serotype 5 (Ad5). The CREF cells display a near diploid karyotype, do not crisscross at confluency, can be maintained at confluency for greater than 7wk at 36 C, and do not form macroscopic colonies when seeded in agar. Transformed cells are identified by their altered morphology and the presence of adenovirus DNA sequences in the transformants, which can be demonstrated by lysing cells directly on nitrocel-

lulose filters and hybridizing with 32P-labeled Ad5 DNA (spot hybridization). The frequency of transformation at 36 C is approx 2 x 10(-4) with wild-type Ad5 and approx 2 x 10(-3) with the temperature-sensitive mutant H5ts125. Southern blot hybridization analysis indicates that five out of six wild-type Ad5-and six out of six H5ts125-transformed CREF clones isolated at 36 C Contain the complete integrated Ad5 genome. Preliminary characterization of four transformed clones (2 wild-type and 2 H5ts125) indicates that, even though transformation was done in CREF cells (a clonal cell line), they differ in their biological properties, such as saturation density and anchorage dependence. (Author abstract) (31 Refs)

16. MAPPING OF RESTRICTION FRAGMENTS AND TRANSFORMING ABILITY OF ADENOVIRUS 31.

Sawada Y, Yamashita T, Kanda F, Sekikawa K, Fujinaga K
Dept. Molecular Biology, Cancer Res. Inst., Sapporo Medical Coll., Sapporo 060, Japan
Tumor Res (Sapporo); 16:7-17 1981

The cleavage maps of adenovirus 31 (Ad31) DNA with five restriction endonucleases and the localization of the transforming ability of the genome is reported. Specific fragments induced by restriction with EcoR1, BamH1, HindIII, SalI, and SmaI were identified via agarose gel electrophoresis. The fragments A and F of EcoR1 digests, the fragments B and E of BamH1 digests and the fragments C and G of HindIII digests were localized at the ends of the Ad31 genome. When the HindIII-C and G fragments were used to transfect subconfluent monolayers of 3Y1 cells, transformed foci became visible 3 wk later in the -G fragment-treated cultures. No transformation occurred in mock-transfected or -C fragment transfected cells. Thus the transforming ability of Ad31 is localized at the end of the genome where HindIII-G maps. (23 Refs)

17. HUMAN LYMPHOMA-LYMPHOMA HYBRIDS AND LYMPHOMA-LEUKEMIA HYBRIDS. I. ISOLATION, CHARACTERIZATION, CELL SURFACE MARKERS, AND B-CELL MARKERS.

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JNCI; 68(2):179-195 1982

Four new somatic cell hybrids were obtained by fusion of various Burkitt's lymphoma (BL)-derived cell lines that had different selective markers: Raji-P3HR-1, Daudi-Raji, and a P3HR-1-P3HR-1 'autohybrid' derived from two P3HR-1 sublines. In addition, a hybrid was was obtained between the Daudi (BL) line and the human leukemia cell line K562. The hybrids were extensively characterized by means of chromosome, isozyme, and HLA surface markers. The phenotypic differences between the parent cell lines allowed some conclusions with respect to the expression of latent Epstein-Barr virus (EBV) genomes, C3 and EBV receptors, and of immunoglobulin and beta 2-microglobulin-HLA expression as well as the influence of the leukemia cell (K562) genome on B-cell properties in the Daudi-K562 hybrid. B-cell and differentiated markers of these hybrids were characterized. High-level expression dominated for the markers C3 and EBV receptors, which showed a good correlation coefficient of 0.84, as was true for Fc receptors and surface immunoglobulin. The Daudi-K562 hybrid showed loss of all B-cell markers but retention of the leukemia cell markers (eg, hemoglobin synthesis). (Author abstract) (59 Refs)

18. HUMAN LYMPHOMA-LYMPHOMA HYBRIDS AND LYMPHOMA-LEUKEMIA HYBRIDS. II. EPSTEIN-BARR VIRUS INDUCTION PATTERNS.

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JNCI; 68(2):197-202 1982

In addition to the spontaneous expression of markers of the early and late phases of the viral cycle [Epstein-Barr virus (EBV), early viral cycle antigens (EA), and viral capsid antigen (VCA)] by a panel of hybrid cell lines derived by fusion of human hematopoietic cell lines, the induction of these markers by three chemical inducers [5-iodo-2'-deoxyuridine, the tumor promoter 12-O-tetradecanoylphorbol 13-acetate (TPA), and sodium butyrate] were analyzed. A variant of the prototype producer of lytic EBV, P3HR-1 (PICAT) was observed to show consistent low spontaneous and induced production of EA and VCA as compared to the P3HR-1 line from which it was derived. An 'autohybrid' (PICATPO-1) between these two lines showed a low production of VCA. Hybrids between Raji and P3HR-1 sublines (RUD-PICAT-1 and RUDPUT-2) showed reduced expression of EBV antigens. Both hybrids were capable of VCA expression, in contrast to the Raji parent that expressed EA but not VCA. A new Raji-Daudi hybrid (DITRUD-1) expressed spontaneous and induced EA and VCA at levels intermediate between its two parents. A different type of hybrid was derived from a Daudi subline and the K562 leukemia cell line (DUTKO-1) and was found to be capable of spontaneous as well as induced EA and VCA expression. Both DUTKO-1 and DITRUD-1 were similar to Daudi in their induction profile in respect to a very low response to TPA. (Author abstract) (20 Refs)

19. TRANSFORMATION OF NIH 3T3 CELLS WITH CLONED FRAGMENTS OF HUMAN CYTOMEGALOVIRUS STRAIN AD169.

Nelson JA, Fleckenstein B, Galloway DA, McDougall JK
Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
J Virol; 43(1):83-91 1982

NIH 3T3 cells were transfected with restriction endonuclease and cloned human cytomegalovirus DNA fragments to identify the transforming region(s). Cleavage of human cytomegalovirus strain AD169 DNA with XbaI and HindIII left a transforming region intact whereas EcoRI inactivated this function. Transfection of cells with cosmids containing human cytomegalovirus DNA spanning the entire genome resulted in transformation by one cosmid, pCM1058, with the AD169 HindIII DNA fragments E, R, T, and a'. Cells were selected for their growth in 1.2% methylcellulose. The clones isolated had a significant replating efficiency and were oncogenic in BALB/c nu/nu mice. Transfection of cosmids and plasmids containing subsets of the viral sequences in pCM1058 identified a common region possessed by all of the transforming recombinant molecules. This region was in the HindIII E fragment with the left boundary defined by the EcoRI d-R junction and the right boundary defined by the HindIII E-T junction. Further mapping and transfection experiments determined that the transforming region was contained within a 2.9-kilobase fragment between map units 0.123 and 0.14 on the prototype molecule of the AD169 strain. (Author abstract) (56 Refs)

20. TRANSPLANTABLE MAREK'S DISEASE LYMPHOMAS. I. GROWTH CHARACTERISTICS DURING DEVELOPMENT IN TWO INBRED LINES OF CHICKENS.

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Avian Dis; 26(2):248-256 1982

Transplantable Marek's disease (MD) lymphomas were developed in G-B1 and G-B2 closely related lines of White Leghorn Chickens differing in major histocompatibility-complex genotypes. Differences in growth patterns between in vivo-passaged tumors that developed persistent transplantability and those that had only limited transplantability were studied, and the proportion of cells in both tumors possessing an MD tumor-associated surface antigen (MATSA) was determined. The most definitive factors in the development of permanently transplantable MD lymphomas appear to be the tumor growth characteristics, associated mortality in syngeneic and allogeneic recipients, and the proportion of lymphoma cells bearing MATSA. The establishment of the present lines, MDCT-UG1 and MDCT-UG2, probably reflected the emergence of highly malignant clones of cells, with subsequent selection of the clones during further passages. Correlated increases in MATSA-positive cells at certain passages of these two tumors suggests that tests for MATSA may be helpful in studies involving development of transplantable MD tumors. Use of these transplantable MD lymphomas is recommended for studies of induction of tumor-specific immunity and studies of the mechanisms by which MD tumors escape from host immune responses. (22 Refs)

21. JMV MAREK'S DISEASE TUMOR CELLS HAVE B-CELL CHARACTERISTICS.

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Dept. Epidemiology and Preventive Medicine, Sch. Veterinary Medicine, Univ. California, Davis, CA, 95616
Clin Immunol Immunopathol; 23(2):527-532 1982

In vivo and in vitro methods were used to determine whether JMV Marek's disease (MD) tumor cells have B-cell or T-cell characteristics. JMV tumor cells were treated with anti-T-cell serum (ATS) and complement (C) or anti-B serum (ABS) and C and then inoculated into 1-day-old chickens. Within 2 wk of inoculation, 95% of chickens inoculated with ATS- and C-treated cells died of JMV-induced leukemia, whereas only 10% of chickens inoculated with ABS- and C-treated cells succumbed. This in vivo result indicated that the malignant cells may be B cells. In vitro studies corroborated this conclusion. ABS and C killed 99 to 100% of cultivated JMV cells, whereas little or no killing of JMV cells was achieved by ATS and C, as demonstrated by trypan blue dye exclusion or by release of 51Cr from labeled JMV cells. Furthermore, after staining of JMV cells with fluorescein-conjugated rabbit anti-chicken immunoglobulin, all cells fluoresced, indicating that JMV cells have surface immunoglobulin. With support of these lines of evidence, we conclude that JMV Marek's disease tumor cells have B-cell characteristics. (Author abstract) (14 Refs)

22. CYTOLOGICAL AND CYTOGENETICAL STUDIES ON HUMAN MENINGIOMA.

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Inst. Human Genetics, Univ. Saar, 6650 Homburg, W. Germany
Cancer Genet Cytogenet; 6(3):249-274 1982

Characteristics of meningiomas which make them a model system for the study of human cancer development are described. Meningiomas show a remarkably good correlation between karyotype and histological and growth patterns which particle cultures of the individual tumors develop in vitro. Histological classification of meningiomas is described. In contrast to true brain tumors, meningiomas occur most offten in the fifth and sixth decade of the life and occur twice as frequently in females as in males. Characterization of meningiomas according to their respective karyotype shows a highly significant shift in sex distribution. In tumors with normal karyotypes, the male:female sex ratio is 1:3 while in more aberrant forms the ratio is approx 1:1. Chromosomal indications for virus involvement in the etiology of meningiomas are presented along with immunofluorescence data, induction of virus particles, and demonstration of episomal Simian virus 40 (SV40) DNA in meningiomas. Experimental transformation of meningioma cell cultures and fibroblast cultures of the same patients with SV40 is described. (62 Refs)

23. OF WOODCHUCKS AND MEN: THE CONTINUING STORY OF HEPATITIS B AND HEPATOCELLULAR CARCINOMA.

Johnson PJ, Williams R
Liver Unit, King's Coll. Hosp. Medical Sch., London SE5 8RX, England
Br Med J; 284(6329):1586-1588 1982

Statistical evidence that presence of hepatitis B surface antigen (HBsAg) is associated with high rates of hepatocellular carcinoma is presented. Studies on the relationship between HBsAg and cirrhosis lead to the hypothesis that in susceptible people the hepatitis B virus causes either a healthy carrier state or chronic liver disease depending on the host's immunological reaction to the virus. After many years the virus becomes part of the host genetic material, and in some cases this may lead to malignant change. Potential uses of vaccines against hepatitis B virus or antiviral chemotherapy in carriers are discussed. (22 Refs)

24. CHROMOSOMAL ABERRATIONS IN CHRONIC B-CELL LYMPHOCYTIC LEUKEMIA.

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Cancer Genet Cytogenet; 6(2):171-181 1982

Production, incidence, and types of chromosome aberrations in chronic B-cell lymphocytic leukemia (B-CLL) are reviewed. Of polyclonal B-cell mitogens, Epstein-Barr virus and lipopolysaccharide from E coli have been the most successful for cytogenetic analysis of B-CLL. The mitogenic responses measured by 3H-labeled thymidine incorporation into leukemic cells and the mitotic index are variable. Difficulties associated with calculating reliable incidence figures for chromosomal aberrations in B-CLL are considered. In B-CLL, both numerical and structural aberrations are found. The most common numerical aberration is an extra chromosome 12. Other aberrations include deletion of chromosome 14, translocations, deletions, and other extra chromosomes. Many of the chromosomal abnormalities seen in B-CLL have also been found in other malignant hematopoietic disorders, particularly lymphoproliferative disorders. However, the most common abnormality, the extra chromosome 12, is rare in other disorders. The biological significance and prognostic importance of chromosomal aberrations in B-CLL are discussed. (72 Refs)

25. IS TRANSFORMATION ASSOCIATED WITH AN INCREASED ERROR FREQUENCY IN MAMMALIAN CELLS?

Pollard JW, Harley CB, Chamberlain JW, Goldstein S, Stanners CP
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J Biol Chem; 257(11):5977-5979 1982

Acute starvation of mammalian cells for amino acids results in translational errors that may be detected by two-dimensional polyacrylamide gel electrophoresis. Using this as an assay for error frequency in mammalian cells, we investigated the hypothesis that neoplastic transformation was associated with an increased error frequency which in turn leads to an increased mutation rate and a decreased efficiency of regulatory controls (phenomena of tumor progression). Although we found that transformation was not always associated with an increased level of mistranslation we showed that SV40 transformation increased the level of translational errors in all cell types tested. (Author abstract) (27 Refs)

VIRUS REPLICATION AND EXPRESSION

26. FINE STRUCTURE OF VIRUS CHROMATIN IN THIN SECTIONS OF SV40-INFECTED CELLS: A CYTOCHEMICAL AND AUTORADIOGRAPHICAL STUDY.

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Institut de Recherches Scientifiques sur le Cancer, CNRS, F-94802 Villejuif Cedex, France
J Gen Virol; 59(part2):235-244 1982

The ultrastructural organization of virus chromatin was studied within nuclei prepared from CV1 cells (cultured monkey kidney cells) lytically infected with SV40 virus (simian virus 40) by a procedure which allows a mild loosening of nucleoproteins. In addition to dispersed host components, DNA-containing nucleoplasmic structures could be identified as virus chromatin both standard staining for structure and specific staining for DNA clearly revealed in thin sections the nucleosomal structure of well-extended virus chromatin as well as alignments of virions on host chromatin. In addition, replicating and transcribing virus chromatins were abundant as revealed by high-resolution autoradiography. Therefore, the procedure of loosening of nucleoproteins used in this report also preserves the active SV40 chromatin and allows the in situ visualization in Epon sections of transcription and replication of the virus genome. (Author abstract) (25 Refs)

27. ATTENUATION IN THE CONTROL OF SV40 GENE EXPRESSION.

Hay N, Skolnik-David H, Aloni Y
Dept. Genetics, Weizmann Inst. Science, Rehovot 76100, Israel
Cell; 29(1):183-193 1982

Nuclei and viral transcriptional complexes were prepared from cells infected with simian virus 40 and incubated in vitro in the presence of alpha-32P-UTP. The in vitro elongated viral RNA appeared with a peak of 5S in sucrose gradients and hybridized preferentially to a promoter-proximal region of SV40 DNA. Treatment of infected cells with proflavine led to transcription of elongated RNA, while treatment of cells with 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole, a drug known to enhance premature termination, augmented accumulation of the promoter-proximal RNA. The in vitro elongated RNA produced a major band of 93-95 nucleotides in length in acrylamide gel. This RNA was found to map between the major initiation site at nucleotide 243 and nucleotides 335-337. The significance of these observations with respect to the transcription termination signal and the control of SV40 gene expression is discussed. (Author abstract) (62 Refs)

28. DISCRETE REGIONS OF SIMIAN VIRUS 40 LARGE T ANTIGEN ARE REQUIRED FOR NONSPECIFIC AND VIRAL ORIGIN-SPECIFIC DNA BINDING.

Prives C, Barnet B, Scheller A, Khoury G, Jay G
Dept. Biological Sciences, Columbia Univ., New York, NY, 10027
J Virol; 43(1):73-82 1982

The nondefective adenovirus Type 2 (Ad2)-simian virus 40 (SV40) hybrid viruses, Ad2+ND2 and Ad2+ND4, have been used to determine which regions of the SV40 genome coding for the large tumor (T) antigen are involved in specific and nonspecific DNA binding. Ad2+ND2 encodes 45,000 Mr (45K) and 56,000 Mr (56K) T antigen-related polypeptides. The 45K polypeptide did not bind to DNA, but the 56K polypeptide bound nonspecifically to calf thymus DNA. Ad2+ND4 encodes 60,000 Mr (60K), 66,000 Mr (66K), 70,000 Mr (70K), 74,000 Mr (74K), and 90,000 Mr (90K) T antigen-related polypeptides, all of which bound nonspecifically to calf thymus DNA. However, in more stringent assays, where tight binding to viral origin sequences was tested, only the 90K protein specified by Ad2+ND4 showed specific high affinity for sequences at the viral origin of replication. From these results and previously published experiments describing the SV40 DNA integrated into these hybrid viruses, it was concluded that SV40 early gene sequences located between 0.39 and 0.44 SV40 map units contribute to nonspecific DNA binding, whereas sequences located between 0.50 and 0.63 SV40 map units are necessary for specific binding to the viral origin of replication. (Author abstract) (41 Refs)

29. SYNTHESIS OF PREMATURELY TERMINATED LATE TRANSCRIPTS OF POLYOMA VIRUS DNA IS RESISTANT TO INHIBITION BY 5,6-DICHLORO-1-BETA-D-RIBOFURANOSYLBENZIMIDAZOLE.

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Tumor Virology Lab., Salk Inst., San Diego, CA, 92138
J Gen Virol; 59(part2):367-376 1982

Exposure of mouse kidney cells to 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole (DRB) (75 to 300 uM) during the late phase of infection by polyoma virus resulted in nearly complete (90 to 98%) inhibition of virus RNA synthesis. Sedimentation analysis revealed that, although the synthesis of high mol wt (greater than 10S) virus RNA was inhibited in a manner parallel to that of total virus RNA, the synthesis of small (3S to 7S) virus RNA was inhibited by only 40 to 50% in the presence of DRB. As a result, virus RNA synthesized in the presence of DRB contained a peak at 3S to 7S in addition to residual high mol wt virus RNA. Small virus RNA from either untreated or DRB-treated cells contained three- to six-fold higher levels of transcripts from the DNA fragment which lies between the BamHI and BglI sites (58.0 to 72.2 map units) than from DNA fragments covering the rest of the virus genome. Furthermore, 80% of the small RNA which hybridized to this fragment was complementary to the L strand of virus DNA. These results suggest that L strand transcripts are initiated within the BglI-BamHI DNA fragment and that a portion of these transcripts is prematurely terminated within

several hundred nucleotides of the site(s) of initiation. DRB had little effect on the synthesis of these prematurely terminated RNAs. (Author abstract) (33 Refs)

30. ASPECTS OF THYMIDINE METABOLISM AND FUNCTION IN CULTURED MAMMALIAN CELLS INFECTED WITH HERPES SIMPLEX VIRUS TYPE 1.

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J Gen Virol; 59(part2):223-234 1982

We have investigated aspects of thymidine metabolism and function in cultured mammalian cells infected with herpes simplex virus under conditions in which virus DNA synthesis was either permitted, or was prevented by inhibiting the virus-induced DNA polymerase with phosphonoacetate. In 30 min pulse-labeling experiments the rates of [3H]thymidine transport into cells and its subsequent phosphorylation both reached max values about 6 hr after infection. During the next 17 hr these rates remained relatively constant in the absence of phosphonoacetate but declined to about 50% of the 6 hr value in the presence of this inhibitor. When the radioactive thymidine was present continuously throughout the infection the accumulated intracellular acid-soluble radioactivity reached max values at about 9 hr. In the absence of phosphonoacetate this declined thereafter, but it remained relatively constant when virus DNA synthesis was prevented. This suggests some established equilibrium between the continuing entry of thymidine into cells and its subsequent removal by excretion. Excretion of thymidine-derived radioactivity was initially established by determining the fate of isotopically labeled host cell DNA. During 24 hr of infection about 50% of the host cell DNA was rendered acid-soluble and of this 60 to 70% was excreted from the cells. High pressure liquid chromatographic analysis of the intracellular acid-soluble radioactivity showed only phosphorylated thymidine derivatives, whereas the excreted radioactivity was exclusively in thymidine. Excretion of intracellular radioactive molecules derived directly from [3H]thymidine in the medium was also observed, but only when virus DNA synthesis was inhibited with phosphonoacetate. Simple kinetic studies with the purified virus-induced DNA polymerase showed that a straight line double-reciprocal plot was obtained when dGTP was used as the limiting substrate (Km = 0.8 uM), but that a biphasic plot was obtained when dTTP was limiting. (28 Refs)

31. EXPRESSION OF HUMAN BETA-INTERFERON CDNA UNDER THE CONTROL OF A THYMIDINE KINASE PROMOTER FROM HERPES SIMPLEX VIRUS.

Reyes GR, Gavis ER, Buchan A, Raj NB, Hayward GS, Pitha PM
Dept. Pharmacology and Experimental Therapeutics, John Hopkins Univ. Sch. Medicine, 725 N. Wolfe St., Baltimore, MD, 21205
Nature; 297(5867):598-601 1982

Transcription of human beta-interferon (IFN-beta) gene and the induction of the gene after replacing the normal 5' flanking sequences with a defined promoter segment from another gene were examined. For this purpose the coding portion of the IFN-beta gene was inserted downstream from the promoter sequence and RNA start site of the thymidine kinase (TK) gene from herpes simplex virus type 1. Expression of interferon activity under the control of the viral TK promoter in this chimeric plasmid was demonstrated by microinjection into nuclei of Xenopus oocytes and by transfection into cells followed by superinfection with herpes simplex virus. (32 Refs)

32. INHIBITORY EFFECT OF E-5-(2-BROMOVINYL)-1-BETA-D-ARABINOFURANOSYLURACIL ON HERPES SIMPLEX VIRUS REPLICATION AND DNA SYNTHESIS.

Descamps J, Sehgal RK, De Clercq E, Allaudeen HS
Dept. Pharmacology, Yale Univ. Sch. Medicine, New Haven, CT, 06510
J Virol; 43(1):332-336 1982

The effect of E-5-(2-bromovinyl)-1-beta-D-arabinofuranosyluracil (BVaraU) on herpes simplex virus (HSV) replication was examined and compared with that of E-5-(2-bromovinyl)-2'-deoxyuridine (BVdUrd). The 50% inhibitory dose against HSV type 1 (HSV-1) was 0.1 ug/ml compared with 0.008 ug/ml for BVdUrd; the antimetabolic 50% inhibitory dose of BVaraU ranged from 20 to 95 ug/ml. The addition of 50 ug of BVaraU per ml to HSV-1-infected Vero cells decreased the synthesis of viral and cellular DNA by 37 and 28%, respectively. The 5'-triphosphate (BVaraUTP) competed with dTTP in DNA synthesis by the herpes-viral and cellular DNA polymerases; the apparent Ki values of HSV-1 DNA polymerase, DNA polymerase alpha, and DNA polymerase beta were 0.14, 0.32, and 5 uM, respectively. Thus, BVaraU was a less effective antiherpesvirus agent than BVdUrd; unlike BVdUrd, it did not appear to be internally incorporated into replicating DNA in virus-infected cells. (Author abstract) (13 Refs)

33. POLYSOME-ASSOCIATED PROTEINS IN HERPES SIMPLEX VIRUS-INFECTED CELLS.

Bartkoski MJ
Dellen Lab., Omaha, NE, 68134
J Virol; 43(1):357-360 1982

In the cytoplasm of eucaryotic cells, mRNA is associated with proteins. These mRNA-protein complexes, termed messenger ribonucleoprotein (mRNP) particles, are divided into two functional classes. The first class contains free (non-ribosome-associated) mRNPs which have been termed informosomes by others. The second class of mRNPs, those associated with polysomes, are actively engaged in protein synthesis and are termed polysomal mRNPs. The experiments described in this paper examined the proteins associated with polyribosomes in uninfected and herpes simples virus Type 1-infected cells. The data indicate that after infection with herpes simplex virus Type 1, specific changes occur in the proteins which normally are found associated with these polysomal mRNPs. These changes include both the appearance of new and possibly virus-specific proteins and the loss of normal host-specific proteins. The relationship of these changes to the patterns of protein synthesis in these cells is also discussed. (Author abstract) (36 Refs)

34. DEFINITION OF A SERIES OF STAGES IN THE ASSOCIATION OF TWO HERPESVIRAL PROTEINS WITH THE CELL NUCLEUS.

Knipe DM, Spang AE
Dept. Microbiology and Molecular Genetics, Harvard Medical Sch., Boston, MA, 02115
J Virol; 43(1):314-324 1982

We examined the kinetics and the nature of the association of two herpes simplex virus proteins, the major DNA-binding protein (ICP8) and the major capsid protein (ICP5), with the nuclei of infected cells. We defined a series of stages in the association of the ICP8 protein with the cell nucleus. (i) Immediately after synthesis, the protein was found in the cytoplasmic

fraction but associated rapidly with the crude nuclear fraction. (ii) The initial association of ICP8 with the crude nuclear fraction was detergent sensitive but DNase resistánt. (iii) At intermediate times, a low level of an intermediate form was observed in which the association of ICP8 with the nuclear fraction was resistant to both detergent and DNase treatment. The protein may be bound to the nuclear matrix at this stage. Inhibition of viral DNA synthesis caused the DNA-binding protein to accumulate in this form. (iv) At late times during the chase period, the association of ICP8 with the cell nucleus was resistant to detergent treatment but sensitive to DNase treatment. Our results argue that at this stage ICP8 was bound to viral DNA. Thus, nuclear association of the DNA-binding protein did not require viral DNA replication. More important is the observation that there is a series of stages in the nuclear association of this protein, and, thus, there may be a succession of binding sites for this protein in the cell during its movement to its final site of action in the nucleus. The major capsid protein showed some similar stages of association with the cell nucleus but the initial association with the nucleus followed a lag period. Its early association with the crude nuclear fraction was also detergent sensitive but was resistant to detergent treatment at later times. Its association with the cell nucleus was almost completely resistant to DNase treatment at all times. Inhibition of viral DNA replication blocked the nuclear transport of this protein. Thus, these two viral proteins share some stages in nuclear transport, although their requirements for nuclear association are different. (27 Refs)

35. IN VITRO TRANSCRIPTION OF TWO EPSTEIN-BARR VIRUS SPECIFIED SMALL RNA MOLECULES.

Jat P, Arrand JR
Imperial Cancer Res. Fund, Lincoln's Inn Fields, *London* WC2A 3PX, England
Nucleic Acids Res; 10(11):3407-3425 1982

Cloned DNA from the EcoRI J fragment of EBV has been used as template for in vitro transcription experiments using cell-free extracts prepared from HeLa or KB cells. Two EBV specific RNAs each about 175 bases in length were synthesized and nuclease S1 mapping experiments determined that these in vitro products corresponded precisely to the in vivo species obtained from Raji cells. Thee two RNA molecules are transcribed by RNA polymerase III and in common with other pol III-synthesized RNAs the coding sequences contain intragenic control regions. The relative abundance of the two RNAs synthesized in vitro differs from that observed in vivo. (Author abstract) (48 Refs)

36. FILAMENTOUS STRUCTURES ASSOCIATED WITH EPSTEIN-BARR VIRUS-INFECTED CELLS.

Moore CL, Griffith JD, Shaw JE
(c/o Shaw) Cancer Res. Center, Dept. Bacteriology and Immunology, Univ. North Carolina, Chapel Hill, NC, 27514
J Virol; 43(1):305-313 1982

After the onset of Epstein-Barr virus DNA and protein synthesis 10 h after superinfection of Raji cells (a cell line containing Epstein-Barr virus DNA but not producing virus), filamentous structures 25 nm in diameter and 0.2 to 1.4 um in length could be detected in the cell cytoplasm by electron microscopy. These structures banded in metrizamide gradients with viral DNA and proteins, but at a density different from that of virions or nucleocapsids. These filaments, enriched in a 155,000-dalton protein similar in size to a major nucleocapsid protein of Epstein-Barr virus, may represent intermediates in viral nucleocapsid assembly. (Author abstract) (37 Refs)

37. EPSTEIN-BARR VIRUS RNA VIII. VIRAL RNA IN PERMISSIVELY INFECTED B95-8 CELLS.

Hummel M, Kieff E
(c/o Kieff) Dept. Biology, Medicine, Section Infectious Diseases, Kovler Viral Oncology Lab., Univ. Chicago, Chicago, IL, 60637
J Virol; 43(1):262-272 1982

More than 50 RNAs expressed by Epstein-Barr virus late in productive infection have been identified. B95-8 infected cells were induced to a relatively high level of permissive infection with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate. Polyadenylated RNAs were extracted from the cell cytoplasm, separated by size on formaldehyde gels, transferred to nitrocellulose, and hybridized to labeled recombinant Epstein-Barr virus DNA fragments. Comparison of RNAs from induced cultures with RNAs from induced cultures also treated with phosphonoacetic acid to inhibit viral DNA synthesis identifies two RNA classes: a persistent early class of RNAs whose abundance is relatively resistant to viral DNA synthesis inhibition and a late class of RNAs whose abundance is relatively sensitive to viral DNA synthesis inhibition. The persistent early and late RNAs are not clustered but are intermixed and scattered through most of segments UL and US. The cytoplasmic polyadenylated RNAs expressed during latent infection were not detected in productively infected cells, indicating that different classes of viral RNA are associated with latent and productive infection. Non-polyadenylated small RNAs originally identified in cells latently infected with Epstein-Barr virus are expressed in greater abundance in productively infected cells and are part of the early RNA class. (Author abstract) (53 Refs)

38. BOVINE PAPILLOMA VIRUS TRANSCRIPTION: POLYADENYLATED RNA SPECIES AND ASSESSMENT OF THE DIRECTION OF TRANSCRIPTION.

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J Virol; 43(1):59-66 1982

The bovine papilloma virus Type 1 (BPV-1)-specific RNA species were identified in virus-induced bovine warts, hamster tumors, and transformed hamster and mouse cells. In each case two major species were present (1.1 and 1.3 kilobases [kb]). Also two species of 1.6 and 1.8 kb appearing in variable amounts were found. Only in the keratinized periphery of the warts, where virus replication takes place, was it possible to reveal an additional 2-kb RNA species. In this tissue, however, the 1.6-kb species was not detected. The basal part of a bovine wart contained an additional minor, 2.9-kb, BPV-1-specific RNA sequence. By hybridization with purified defined BPV-1 DNA fragments it was shown that most of the coding sequences of the 2-kb species were transcribed from a region between 0.02 and 0.19 map units. The majority of the coding sequences of the smaller species in transformed cells were located in the region between 0.31 and 0.61 map units. The putative 5' ends mapped between 0.72 and 0.96 map units. Oligodeoxythymidylic acid-primed [32P]cDNA was synthesized from various RNA preparations to generate probes for the detection of 3' termini of the polyadenylated BPV-1 RNAs. By hybridization across the BPV-1 genome only one signal between the map positions 0.30 and 0.40 was obtained when RNA from transformed cells and from a tumor was used as a template. In contrast, RNA from the periphery of a wart led to the detection of an additional signal which was confined to the region between 0.96 and 1.00 map units. From the arrangement of both the 3' termini and the coding areas along

the viral genome it appears that several RNA species are transcribed from one DNA strand. (Author abstract) (17 Refs)

39. A HUMAN HEPATOMA CELL LINE (PLC/PRF/5) PRODUCES LUNG METASTASES AND SECRETES HBSAG IN NUDE MICE.

Sattler FR, Paolucci S, Kreider JW, Ladda RL
500 University Drive, Hershey, PA, 17033
Eur J Cancer Clin Oncol; 18(4):381-389 1982

PLC/PRF/5 is a human liver cancer cell line which synthesizes hepatitis B virus surface antigen (HBsAg). These cells produced tumors in 6 of 8 (75%) congenitally athymic nude mice at 13 of 28 subcutaneous injection sites and in 13 of 14 (93%) mice inoculated intraperitoneally. Tumors were successfully transplanted to four of six additional nude mice. Tumor growth was rapid. Growth of cephalad tumors was significantly greater than for caudal tumors (0.39 mm/day vs 0.28 mm/day). Microscopic examination of tumors showed moderately well-differentiated hepatocellular carcinoma. Foci of identical cells were present in pulmonary veins in 7 of 14 tumor-bearing animals. Tumor cell karyotype was identical with that of PLC/PRF/5 cells. In addition, HBsAg was detectable in high titer in animals with extensive tumor. Biological features of PLC/PRF/5-induced tumors in nude mice appeared to closely resemble human hepatocellular carcinoma. Moreover, HBsAg may provide a marker of tumor growth. (Author abstract) (24 Refs)

VIRAL GENETICS

40. ISOLATION AND CHARACTERIZATION OF DEFECTIVE SIMIAN VIRUS 40 GENOMES WHICH COMPLEMENT FOR INFECTIVITY.

O'Neill FJ, Maryon EB, Carroll D
Dept. Cellular, Viral and Molecular Biology, Univ. Utah, Salt Lake City, UT, 84148
J Virol; 43(1):18-25 1982

A new variant of simian virus 40 (EL SV40), containing the complete viral DNA separated into two molecules, was isolated. One DNA species contains nearly all of the early (E) SV40 sequences, and the other DNA contains nearly all of the late (L) viral sequences. Each genome was encircled by reiterated viral origins and termini and migrated in agarose gels as covalently closed supercoiled circles. EL SV40 or its progenitor appears to have been generated in human A172 glioblastoma cells, as defective interfering genomes during acute lytic infections, but was selected during the establishment of persistently infected (PI) green monkey cells (TC-7). PI TC-7/SV40 cells contained EL SV40 as the predominant SV40 species. EL SV40 propagated efficiently and rapidly in BSC-1, another line of green monkey cells, where it also formed plaques. EL SV40 stocks generated in BSC-1 cells were shown to be free of wild-type SV40 by a number of criteria. E and L SV40 genomes were also cloned in the bacterial plasmid pBR322. When transfected into BSC-1 cell monolayers, only the combination of E and L genomes produced a lytic infection, followed by the synthesis of EL SV40. However, transfection with E SV40 DNA alone did produce T-antigen, although at reduced frequency. (Author abstract) (28 Refs)

IMMUNOLOGICAL STUDIES

41. PROTECTION OF NEONATAL MICE AGAINST HERPES SIMPLES VIRUS INFECTION: PROBABLE IN VIVO ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY.

Kohl S, Loo LS
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J Immunol; 129(1):370-376 1982

Infant mice are extremely susceptible to fatal Herpes simplex virus (HSV) infection. They are unable to produce antibody to HSV, and their leukocytes cannot mediate antibody-dependent cellular cytotoxicity (ADCC) to HSV-infected cells. In order to avoid H-2-dependent effector mechanisms and instead analyze possible in vivo ADCC, a murine model employing adoptive transfer of antibody and human leukocytes was developed. Administration of either human immune globulin or leukocytes ip from HSV immune or nonimmune humans could not protect infant C57BL/6 mice from fatal HSV infection. In contrast, a combination of a subneutralizing dilution of globulin and leukocytes from nonimmune or immune human donors, given 1 day before inoculation, was highly protective against lethal HSV infection. The cells involved included lymphocytes or monocyte-macrophages. At least 5 x 10(6) viable leukocytes (or 1 x 10(6) monocyte-macrophages) and immune serum globulin concentrations as low as 10(-8) were protective. Infected cell monolayer adsorption and DEAE column fractionation demonstrated that the protection by globulin was due to specific antiviral IgG antibody. Protection was not seen in animals receiving virus before immune transfer. Protection did not involve synergistic viral neutralization by antibody and cells, as shown by in vitro experiments. Animals receiving globulin and cells, unlike normal infant mice, had circulating antiviral antibody and peritoneal leukocytes able to mediate ADCC to HSV-infected cells. This is the first in vivo evidence for the role of human ADCC. This model also allows for the in vivo evaluation of the ability of cells from immunocompromised humans to curb viral infection. (Author abstract) (72 Refs)

42. EPSTEIN-BARR VIRUS ANTIGENS -- A CHALLENGE TO MODERN BIOCHEMISTRY.

Thorley-Lawson DA, Edson CM, Gellinger K
Dept. Medicine, Div. Geographic Medicine, Tufts Univ. Medical Sch., Boston, MA, 02111
Adv Cancer Res; 36:295-348 1982

The serologically defined antigens of Epstein-Barr virus (EBV) are reviewed, including transformation antigens, early antigens, and late antigens. EBV nuclear antigens; lymphocyte-determined membrane antigens; intracellular early antigens and early membrane antigens; late membrane antigens and viral capsid antigens; the molecular biology of antigens and the polypeptides; and immune responses to viral antigens are discussed. Of the 50 or more polypeptides that could theoretically be coded by the 85-90 x 10(6) mol wt of unique DNA in EBV, 20-25 polypeptides are known. Defining the specificity and function of EBV for various antigens will require use of a permissive in vitro system. Since this has not been discovered, the use of monoclonal reagents seems to be the most promising alternative. The use of the cottontop marmoset as an experimental subject for elucidating the role of neutralizing antibodies in preventing the establishment of infection may be defended in terms of the animal's lack of immune responsiveness. Its suitability for studies of infection establishment and the usefulness of studies of isolation of individual membrane antigen compo-

nents, a goal which is currently not feasible, are discussed. Viral subunit vaccination in primates is the first step in understanding the the interaction of EBV with the immune response during infection, which may eventually lead to preventive measures in man. (212 Refs)

43. LEUKOCYTE MIGRATION INHIBITION INDUCED BY EPSTEIN-BARR VIRUS ANTIGENS. IMMUNITY IN INFECTIOUS MONONUCLEOSIS AND SOME IMMUNODEFICIENT AND IMMUNOSUPPRESSED STATES.

Szigeti R, Klein E, Masucci MG, Ernberg I, Svedmyr E, Klein G
II. Gyermekklinika, Semmelweis Orvostudomany Egyetem, Budapest, Hungary
Orv Hetil; 123(20):1217-1221 1982

Leukocyte migration inhibition (LMI) elicited by Epstein-Barr virus (EBV) antigens was studied in 13 patients with infectious mononucleosis (IM), 2 patients with chronic mononucleosis, 4 with sex-linked lymphoproliferative disease and in 12 patients with neoplastic diseases (5 with Hodgkin's disease, 6 with non-Hodgkin's lymphoma and 1 with chronic lymphocytic leukemia). Appreciable LMI was observed in the patients with IM in response to enriched early antigen-virus capsid antigen (EA/VCA) extracts. Similar LMI of mononucleosis type (sensitivity to EA/VCA and insensitivity to EBV nuclear antigen (NA)) was seen in chronic mononucleosis as well, while the patients with sex-linked lymphoproliferative disease failed to respond to any EBV antigens. Tumor patients with elevated EBV antibody titers either showed LMI similar to that seen in healthy, seropositive controls or LMI of the mononucleosis type. The findings suggest that an intact immune system is necessary for LMI to occur in response to EBVNA or to crude cellular extracts containing mainly EBVNA, while sensitivity to EA/VCA is present when the immune system is impaired to a certain extent. Late hypersensitivity reaction in vitro is absent in severe immunodeficiency or immunosuppression. (42 Refs) (In HUN)

44. CYTOTOXIC T CELLS IN CYTOMEGALOVIRUS INFECTION. HLA-RESTRICTED T-LYMPHOCYTE AND NON-T-LYMPHOCYTE CYTOTOXIC RESPONSES CORRELATE WITH RECOVERY FROM CYTOMEGALOVIRUS INFECTION IN BONE-MARROW-TRANSPLANT RECIPIENTS.

Quinnan GV, Kirmani N, Rook AH, Manischewitz JF, Jackson L, Moreschi G, Santos GW, Saral R, Burns WH
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N Engl J Med; 307(1):7-13 1982

Prospective studies were conducted of cell-mediated cytotoxic response in 58 recipients of syngeneic or allogeneic bone-marrow grafts. Emphasis was placed on the period 4-12 wk after transplantation, when the risk of cytomegalovirus (CMV) infection is greatest. Forty-three patients had CMV during the period of study; 23 patients survived infection. CMV infection was the principal cause of death in 12 patients, and an additional 8 patients died from other causes during acute CMV infection. A significant rise in cytotoxicity was observed early in the course of infection, as measured by CMV-specific cytotoxic lymphocyte response. Evidence of CMV-specific cytotoxic response was found in all patients who survived infection. Patients with nonfatal infections showed significantly greater response than those with fatal infections. The natural-killer-cell activity of lymphocytes from patients with nonfatal infections was within the normal range both before and after the onset of infection, but in the group with fatal infection, lower than normal activity was observed in 4 patients before the onset of infection and in 11 patients afterward. Neither the presence nor level of complement-fixing antibodies in donors or recipients before transplantation correlated with the risk of infection or the likelihood of recovery. The finding that the capacity for cytotoxic response, mediated both by HLA-restricted T lymphocytes and nonrestricted non-T lymphocytes during CMV infection, correlated with recovery strongly suggests that these effector cells mediate recovery from CMV infection. (32 Refs)

45. PREVENTION OF SYNGENEIC TUMOR GROWTH IN VACCINIA VIRUS-PRIMED MICE BY IMMUNIZATION WITH VACCINIA VIRUS-MODULATED TUMOR CELLS.

Wu KS, Ueda S, Sakaue Y, Ohashi Y, Ikuta K, Sugano T, Kato S
Dept. Pathology, Res. Inst. Microbial Diseases, Osaka Univ., 3-1, Yamada-oka, Suita, Osaka 565, Japan
Biken J; 24(4):153-158 1981

Immunization with vaccinia virus-infected and then x-ray-irradiated murine hepatoma MH134 cells provided C3H/He mice with strong resistance to challenge with viable MH134 cells. Male C3H/He mice of 5 to 6 wk old were primed intraperitoneally (IP) with 1 x 10(7) PFU of live vaccinia virus (Ikeda strain) after irradiation with 250 R of x-ray. Three wk after priming, the mice were immunized IP three times at weekly intervals with 1 x 10(7) x-ray-irradiated MH134 cells that had been infected with vaccinia virus 8 hr before irradiation. Over 60% of these cells showed vaccinia virus-induced antigen on their surface (membrane antigen). Challenge with viable MH134 cells was done by inoculating 1 x 10(5) cells IP 1 wk after immunization. During a 4-wk observation period, all the untreated control mice died with ascites. On the contrary, all the mice that were x-ray-irradiated, primed and immunized survived challenge with the tumor cells for at least 4 wk. The mortalities of mice in other groups that were not irradiated, or not primed, or immunized with only x-ray-irradiated tumor cells, were at lowest 50%. (Author abstract) (14 Refs)

46. ISOLATION AND CHARACTERIZATION OF SIMIAN VIRUS 40 EARLY REGION DELETION MUTANTS.

Sompayrac LM, Danna KJ
Dept. Molecular, Cellular and Developmental Biology, Univ. Colorado, Boulder, CO, 80309
J Virol; 43(1):328-331 1982

We constructed a tsB4/dl884 double-mutant helper virus and used it to isolate two simian virus 40 early region deletion mutants that lack about half of the DNA sequences normally used to encode the large tumor antigen (T). Both mutants make a normal-sized small t antigen, but neither mutant can replicate its DNA in the absence of a T+ helper. (Author abstract) (15 Refs)

47. MAPPING OF A DNA-BINDING DOMAIN OF SIMIAN VIRUS 40 T-ANTIGEN USING NON-DEFECTIVE ADENOVIRUS 2-SIMIAN VIRUS 40 HYBRID VIRUSES.

Montenarh M, Deppert W, Henning R
Dept. Biochemistry, Univ. Ulm, P.O. Box 4066, 7900 Ulm, W. Germany
FEBS Lett; 142(1):129-132 1982

In the attempt to localize a nonspecific DNA-binding region in the polypeptide chain of tumor antigen (T-Ag), a comparison was made between the calf thymus DNA-binding properties of simian virus 40 (SV40) T-Ag from SV40-transformed

human cells and T-Ag related polypeptides coded for by Ad+ND2 and Ad2+ND4. The study suggests a DNA-binding domain inside the polypeptide chain of T-Ag located between maximally 0.53 and 0.39 SV40 DNA map units. (21 Refs)

OTHER RELATED STUDIES

48. COVALENT AFFINITY LABELING BY PERODATE-OXIDIZED [ALPHA-32P]ATP OF THE LARGE-T PROTEINS OF POLYOMA AND SV40 VIRUSES.

Clertant P, Cuzin F
Centre de Biochimie du Centre National de la Recherche Scientifique, Universite de Nice, Parc Valrose, 06034 Nice, France
J Biol Chem; 257(11):6300-6305 1982

Incubation of crude extracts from cells lytically infected with polyoma virus in the presence of periodate-oxidized [alpha-32P]ATP led to the radioactive labeling of one main polypeptide immunoprecipitated by anti-T antigen antibodies. It was absent from extracts of mock-infected cells and exhibited an apparent Mr value of 105,000 identical with that of the large-T viral protein. This polypeptide was unambiguously identified as large-T on the basis of the heat sensitivity of the in vitro labeling in extracts from cells infected with a tsa mutant. The amount of incorporated radioactivity was found to increase linearly with that of infected cell extract and with the incubation time. Labeling resulted from the specific binding of an oxidized ATP molecule to a nucleotide binding site of the large-T protein, since it was efficiently competed by unlabeled ATP. Study of the dependence on the concentration of oxidized ATP evidenced a first order kinetic for the labeling reaction. Similar results were obtained for the large-T protein of SV40 virus. (Author abstract) (32 Refs)

49. EFFECTS OF BENZO[A]PYRENE DIOL EPOXIDE ADDUCTS ON DNA SYNTHESIS IN MAMMALIAN CELLS.

Rinaldy A, Lockhart ML, Deutsch JF, Ungers GE, Rosenberg BH
Walker Lab., Sloan-Kettering Inst., Rye, NY, 10580
Mutat Res; 94(2):383-391 1982

Effects of benzo[a]pyrene diol epoxide (BPDE) adducts in DNA were studied in CV1-P cells infected with SV40 virus treated with BPDE in vitro prior to infection. Replication in CV-1 monkey kidney cells of SV40 DNA containing an av of 11 anti-BPDE adducts/molecule were compared to that of similar DNA containing no adducts. The decreased replication of DNA from BPDE-treated virus may have resulted directly from the presence of BPDE adducts in the DNA, or it may have been due to a decrease in cellular uptake or uncoating of the virus, possibly caused by the presence of BPDE adducts in the viral capsid proteins. Although replication of the DNA of the BPDE-treated virus was initially low, in the course of time the ratio of treated tsA to deletion molecules approached that of the mock-treated control. Viruses with 11 adducts/DNA molecule were only approx 12% viable in spite of the fact that most or all of the DNA molecules are ultimately able to replicate. The loss of viability thus suggested either a persistent defect in transcription of the infecting DNA, caused by BPDE adducts that do not prevent replication and/or a high probability of producing defective progeny molecules during repair or bypass replication of parental molecules containing BPDE adducts. The 12% viability indicated an av of approx 2/11 lethal hits. Data for three adducts/molecule indicated that approx 7% of the adducts were lethal. Results implied that a minor adduct might be responsible for the the loss of viability. (19 Refs)

50. DIFFERENTIAL EFFECTS OF HYDROXYUREA ON THE SURVIVAL OF UV- AND MNNG-TREATED ADENOVIRUS 5.

Day RS, Ziolkowski CH
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Mutat Res; 94(2):257-262 1982

Effects of hydroxyurea (HU) on the survival of both UV- and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) treated adenovirus 5 when used to infect either human cell strains XP12BE, CRL1220, XP4BE, and KD were studied and observed that HU decreased in all cases studied the survival of UV-irradiated adenoviruses. A 24-hr 10-milliM (mM) HU post-infection treatment of XP12BE fibroblasts caused apparent increase in sensitivity of UV-irradiated adenovirus 5. The magnitude of inhibition of viral recovery by HU, however, was less than that observed when infecting normal fibroblasts. In the presence of HU, the lethality of a given dose was increased by a factor of 2.1 in normal, cells, and by 1.29 using the XP12BE strain. In ancillary experiments, 10 mM HU present with KD cells only during the 24 hr prior to infection caused no enhancement of viral inactivation. HU (10 mM) applied to CRL1187 or KD normal fibroblasts (Mer+) infected with MNNG-treated adenoviruses caused no change in viral survival indicating that HU dose not interfere greatly with the Mer repair pathway. However, HU treatment did increase the survival of MNNG-treated viruses when infecting the A1336 and A172 Mer- strains, the lethality of a given dose being increased by 1.52 +-0.10. The result showed that viral recovery occuring independently of the Mer system is somewhat susceptible to blockage by the HU treatment. (26 Refs)

51. POTENT ANTIVIRAL 2'-FLUORO-ARABINOSYL PYRIMIDINE NUCLEOSIDES: LACK OF MUTAGENIC ACTIVITY.

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Carcinogenesis; 3(5):593-596 1982

The carcinogenicity of many drugs, such as antitumor agents, is a subject of growing concern. The newly developed pyrimidine nucleosides, 2'-fluoro-5-iodo-1-beta-D-arabinofuranosylcytosine (FIAC) and 2'-fluoro-1-beta-D-arabinofuranosyl-5-methyluracil (FMAU), have shown potent anti-herpes virus activity in tissue cultures, laboratory animals and man and an activity to inhibit the growth of certain tumor cell lines in vitro. Radioactivity of 14C-labeled FIAC and FMAU is incorporated into the DNA of normal and neoplastic mammalian tissues. However, we now report that FIAC and FMAU are inactive in a bacterial mutagenesis assay (Salmonella-microsome test) and in a mammalian cell mutagenesis assay employing V79 Chinese hamster cells in vitro. Both agents did not induce unscheduled DNA synthesis in primary Wistar rat hepatocytes in vitro. (Author abstract) (7 Refs)

52. VIRAL HEPATITIS. 1981 INTERNATIONAL SYMPOSIUM.

Szmuness W, Alter HJ, Maynard JE

Lab. Epidemiology, Lindsley F. Kimball Res. Inst., New York Blood Center, New York, NY

Viral Hepatitis. 1981 International Symposium. Philadelphia, The Franklin Institute Press, 843 pp., 1982.

Previously unpublished reports concerning viral hepatitis were presented and discussed. Topics ranged from molecular biology to vaccine development, from hepatitis virus culture to the treatment of clinical disease, from hepatoma in the woodchuck to the association of hepatitis B virus (HBV) infection with hepatocellular carcinoma in man. New insights into the molecular anatomy of hepatitis A and B viruses were presented and the in vitro propagation of whole virus or virus-related proteins was described. DNA viruses termed 'hepadna' include human HBV and related viruses found in the woodchuck, ground squirrel, and Pekin duck. The high occurrence of hepatocellular carcinoma among animals infected with HBV-like viruses provided strong virologic support for the epidemiologic data that suggests a causal relationship between HBV infection and the development of human hepatocellular carcinoma. The epidemiology, diagnosis, pathogenesis, and chronic sequelae of hepatitis B and the evolving clinical spectrum of non-A, non-B hepatitis were discussed. Passive and active immunization programs in Australia, Japan, England, Holland, Senegal, and the United States were reported. Overall, the subunit vaccine was safe, highly immunogenic, and almost completely protective among persons who responded with development of anti-surface antibody. Abstracts and summaries of 14 workshops are presented. Unresolved issues included measures to prevent intrahospital transmission of HBV, reduction of post-transfusion hepatitis, use of corticosteroids for treatment of chronic hepatitis, and the use of hepatitis B immunoglobulin in the prevention of type B hepatitis. (1302 Refs)

53. ACTIVITY OF 2-FLUORO-5-METHYLARABINOFURANOSYLURACIL AGAINST MOUSE LEUKEMIAS SENSITIVE TO AND RESISTANT TO 1BETA-D-ARABINOFURANOSYLCYTOSINE.

Burchenal JH, Chou TC, Lokys L, Smith RS, Watanabe KA, Su TL, Fox JJ

Memorial Sloan-Kettering Cancer Center, New York, NY, 10021

Cancer Res; 42(7):2598-2600 1982

A new pyrimidine nucleoside, 2'-fluoro-5-methyl-1-beta-D-arabinofuranosyluracil, previously has been shown to be active against the herpes group of viruses in vitro and in vivo. It is also active against mouse and human leukemic cells in culture and against mouse leukemias L1210, P388, and P815 in vivo. In contrast to other 1-beta-D-arabinofuranosylcytosine (ara-C) derivatives, 2'-fluoro-5-methyl-1-beta-D-arabinofuranosyluracil, when given either ip or po, is highly active against lines of leukemias P815 and L1210 made resistant to ara-C. Against P815/ara-C and L1210/ara-C, it is more effective than is 5-azacytidine, a drug which has shown definite effectiveness in patients with acute leukemia whose disease has become resistant to ara-C. For these reasons, 2'-fluoro-5-methyl-1-beta-D-arabinofuranosyluracil would seem to merit clinical trial in patients with acute nonlymphocytic leukemia whose disease has become resistant to ara-C. (Author abstract) (10 Refs)

54. INHIBITION OF 7,12-DIMETHYLBENZ(A)ANTHRACENE-INDUCED TUMORS IN SYRIAN HAMSTERS BY PRIOR INFECTION WITH H-1 PARVOVIRUS.

Toolan HW, Rhode SL, Gierthy JF

Inst. Medical Res. Bennington, Bennington, VT, 05201

Cancer Res; 42(7):2552-2555 1982

Hamsters, given injections, sc at birth of H-1 parvovirus and 1 mo later given a single injection of 7,12-dimethylbenz(a)anthracene, had a 38% tumor incidence compared with a 95% incidence in animals receiving 7,12-dimethylbenz(a)anthracene alone. Thus, H-1 which, it has already been shown, invokes a resistance to the incidence of spontaneous and adenovirus-induced neoplasms in hamsters also produces a suppression of a carcinogen-induced tumor in these animals; this suggests that the H-1-induced barrier to successful oncogenesis by these diverse agents has a common mechanism which, present experiments indicate, is not related to a positive or negative H-1 serology. The pathology of the 7,12-dimethylbenz(a)anthracene-induced tumors was similar for both control and H-1-infected hamsters. Although all but one of the primary neoplasms were anaplastic fibrosarcomas as reported previously by others, 25% of the affected females had, in addition, mammary adenocarcinomas, an extremely rare tumor in hamsters. (Author abstract) (26 Refs)

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VIRUS ISOLATION/ STRUCTURE/ CHARACTERIZATION/ IDENTIFICATION

1. STRUCTURE OF ADENOVIRUS NUCLEOPROTEIN CORE STUDIED BY CIRCULAR DICHROISM AND SELECTIVE RADIOCHEMICAL LABELING.

Boulanger P, Loucheux-Lefevbre MH
Unite de Virologie Moleculaire de l'INSERM, U. 233, Place de Verdun, 59045 Lille Cedex, France
Biochem Biophys Res Commun; 107(2):470-480 1982

The conformational structure of wild-type adenovirus type 2 viral DNA (AV-DNA) within intact virion and adenovirus cores of different protein contents was studied by circular dichroism (CD) and by selective radiochemical labeling of some critical amino acids. Virus was grown on KB cells and was extracted in CsCl gradients. CD spectra were compared in intact virion and in deoxycholate and sarcosyl cores. AD-DNA was in highly condensed and packed conformation in both intact virus particles and deoxycholate cores, as found in chromatin nucleosomes. There were minor but significant CD spectral differences with eukaryotic chromatin which indicated that the adenovirus nucleosome-like structures had specific

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conformational features. AD-DNA complexed with protein VII in sarcosyl cores elicited a CD spectrum reminiscent of a relaxed DNA, which suggests that the adenovirus sarcosyl cores were no longer organized in nucleosome subunits. In parallel in vitro studies, the nature of the bonds involved in core structure was studied by selective labeling of some critical amino acids. The accessibility of lysyl and tyrosyl residues in adenovirus core proteins was investigated with the use of 14C-dansyl as a probe. Results suggest that core protein V was associated with protein VII mainly via hydrophobic bonds, and had a major role in the maintenance of the nucleosomal structure of the viral core. (34 Refs)

2. HYPOMETHYLATION OF HELA CELL DNA AND THE ABSENCE OF 5-METHYLCYTOSINE IN SV-40 AND ADENOVIRUS (TYPE 2) DNA: ANALYSIS BY HPLC.

Diala ES, Hoffman RM
Dept. Pediatrics, M-009, Univ. California at San Siego Sch. Medicine, La Jolla, CA, 92093
Biochem Biophys Res Commun; 107(1):19-26 1982

The extent of methylation in highly purified simian virus 40 (SV40) and adenovirus (type 2) DNA was investigated using a sensitive high pressure liquid chromatographic procedure. 5-Methylcytosine was not detected in virion SV40 DNA. This lack of methylation in SV40 contrasts to that in monkey kidney cells in which SV40 is propagated, in which 3.7% of all cytosines are methylated. 5-Methylcytosine was also not detected in adenovirus (type 2) DNA bases. In HeLa cells in which adenovirus is grown, 2.3% of cytosines were found to be methylated. This level of methylation is considerably lower than in normal and SV40-transformed human cells, which is about 3%. The contribution of DNA methylation to SV40 and adenovirus gene activity is discussed. (31 Refs)

3. STABILITY AND COMPONENTS OF MATURE SIMIAN VIRUS 40.

Bina M, Beecher S, Blasquez V
Dept. Chemistry, Div. Biochemistry, Purdue Univ., West Lafayette, IN, 47907
Biochemistry; 21(13):3057-3063 1982

We have examined the stability of mature simian virus 40 (SV40) to changes in pH and have investigated the DNA-specific enzymatic activities that are released from SV40 virions. Our studies show that when SV40 virions are disrupted by the alkaline disruption method described by Brady et al, In addition to the capsid proteins, a DNA topoisomerase and a DNA endonuclease are released. Treatment with a reducing agent and divalent ion chelator is required for the release of both enzymes from the mature virions. Kasamatsu and Wu have previously isolated from SV40 virions a protein covalently linked to SV40 DNA under strongly denaturing conditions. Considering the known biochemical properties of DNA topoisomerases, we propose that the protein in Kasamatsu and Wu's isolated protein-DNA complex is the SV40-associated topoisomerase. In this work, we show that the topoisomerase is activated by Mg++ ions and does not require a high-energy cofactor to relax supercoiled DNA. On the other hand, the endonuclease is active at 37 C only in the presence of Mg++ ions. While the topoisomerase and the endonuclease activities are released from the virions near neutral conditions, the extent of dissociation of capsid proteins from SV40 chromosomes and the shape of subviral DNA-protein complexes depend on pH. At pH 10.4, the DNA-containing particles sediment at 60 S. At lower pH values, 9.8 and 9.2, the subviral nucleoprotein complexes sediment at 75 and 92 S, respectively. Below pH 9.0, the virus particles decay to a heterogeneous mixture of DNA-containing particles. The majority of the nucleoprotein complexes that result from disruption of the virions at pH 8.8 appears as highly compact SV40 chromatin. Electron microscopic observations and biochemical analysis suggest that a fraction of encapsidated SV40 chromatin seems to be deficient in histones or 'normal' nucleosomes. (Author abstract) (39 Refs)

4. EFFECT OF NUCLEASE S1 ON DNA OF HIGHLY ONCOGENIC SIMIAN ADENOVIRUS SA7(C8).

Zavizion BA, Naroditskii BS, Grodnitskaia NA, Makhov AM, Tikhonenko TI
D. I. Ivanovskii Inst. Virology, Moscow, USSR
Mol Biol (Mosk); 16(3):569-574 1982

Comparative evaluation of the effects of nuclease S1 on DNA and on complex of DNA with terminal protein of adenovirus SA7 showed the cleavage of the terminal protein only after preliminary hydrolysis. Treatment with nuclease S1 resulted in 5-10x decrease of the infectivity of DNA. (22 Refs) (In RUS)

5. PROPERTIES OF THE GENOME OF EQUINE HERPESVIRUS TYPE 3.

Atherton SS, Sullivan DC, Dauenhauer SA, Ruyechan WT, O'Callaghan DJ
(c/o O'Callaghan) Dept. Microbiology, Univ. Mississippi Medical Center, Jackson, MS, 39216
Virology; 120(1):18-32 1982

Methods developed for the isolation and purification of equine herpesvirus Type 3 (EHV-3) DNA were shown to yield quantities of intact infectious DNA molecules suitable for characterization by physicochemical, biochemical and electron microscopic methods. Preparations of EHV-3 DNA were shown by CsCl analytical ultracentrifugation to be comprised of a single species of DNA. Rate velocity centrifugation studies revealed that the EHV-3 DNA sedimentation coefficient corresponds to a mol wt value of 90 to 100 megadaltons (Md). Intact viral DNA molecules were found to be 96 to 100 Md by electron microscopy, and restriction enzyme analyses with BamHi, EcoRI, and HindIII yielded an av mol wt of 96.2 Md. Results indicated that a set of DNA sequences located at one terminus is repeated within the genome and that two populations of molecules exist with regard to the orientation of these repeat sequences. Electron microscopic examination of reannealed EHV-3 DNA molecules revealed structures that contained a single-strand loop equivalent to a duplex mol wt of 5.3 Md at one end of the molecule contiguous to a double-strand region which terminated in a long single-strand segment. These structures were identical in morphology to those observed for EHV-1 DNA which has an overall genomic structure of an L (long) region covalently linked to an S (short) region; the S region consists of a unique segment (Us) bracketed by inverted repeat sequences. The above findings indicate that the 96.2-Md EHV-3 genome is comprised of an L region of approx 73-76 Md covalently linked to a 20-to 23-Md S region; the S region contains a 5.3-Md Us segment bounded by 8-Md inverted repeat sequences that allow the S region to invert relative to the fixed L region and the genome to exist in two isomeric arrangements. (57 Refs)

6. TRANSMISSION AND SCANNING ELECTRON MICROSCOPIC STUDIES OF HERPES VIRUSES.

Nii S
Dept. Virology, Okayama Univ. Medical Sch., Okayama, Japan
Excerpta Med Int Congr Ser; 571:3-11 1982

Electron microscopic (EM) studies of various herpesviruses (HPVs) are presented: ultrastructure of virions of HPV, morphological variability of intranuclear virus particles correlated with their development, variable appearance of viral cores among members of the HPV family, envelopment, particles observed on the surface of cells infected with HPVs, and time-sequence studies on the appearance of viral particles on the surface of cells infected with herpes simplex virus (HSV). From a time-sequential study and EM observations of cells infected with several HVPs, a hypothesis is proposed concerning the assembly of intranuclear particles. Differences noted in the appearance of viral cores of different HVPs (Varicella-zoster virus, HPV of turkey [HVT], human cytomegalovirus [HCMV]) indicate inherent differences in their ultrastructure. In vitro cultured cells infected with various HPVs examined by scanning electron microscopy showed particles 170-200 nanometer in diameter in 5/8 HPV examined: HSV-1, HSV-2, VZV, HCMV and infectious laryngo-tracheitis virus. In the time-sequence studies, many virions of HSV attached to the surface of infected cells. The mechanism of this phenomenon appears to be closely related with that of egress of the virus. (9 Refs)

7. MOLECULAR STRUCTURE OF HERPESVIRUS PAPIO DNA AND CLONING OF ITS REPEAT SEQUENCE IN PLASMID PACYC 184.

Lee YS, Nonoyama M, Rabin H
Showa Univ. Res. Inst. for Biomedicine in Florida, 5180 113th Ave. North, Clearwater, FL, 33520
Excerpta Med Int Congr Ser; 571:12-18 1982

Genetic engineering technology was used to analyze the genomes of herpes virus Papio (HVP) DNA. HVP productive cells (594S/F9) were propagated at 37 C. Virus was isolated from productive cells treated with 12-O-tetradecanoylphorbol-13-acetate. Blotting hybridization and double enzyme digestion was used to form an overall linkage map of HVP DNA. Similarity was found between HVP and Epstein-Barr virus (EBV) in both biological and biochemical aspects. Terminal heterogeneity and internal repeat sequence (IR) were unique features in EBV and HVP DNA and was also found in gorilla DNA isolated from a productive lymphoblastoid cell line. It is suggested that IR might be a unique sequence among all primate EBV-like viruses. (15 Refs)

8. ANALYSIS OF YABA TUMOR POXVIRUS-INDUCED PROTEINS IN INFECTED CELLS BY TWO-DIMENSIONAL GEL ELECTROPHORESIS.

Vafai A, Rouhandeh H
(c/o Rouhandeh) Lab. Molecular and Cancer Virology, Dept. Microbiology, Southern Illinois Univ. at Carbondale, Carbondale, IL, 62901
Virology; 120(1):65-76 1982

Lack of inhibition of host protein synthesis by Yaba tumor poxvirus and comigration of viral and host proteins did not interfere with high resolution of virus-induced proteins when two-dimensional gel electrophoresis was employed. At total of 84 virus-induced proteins, 44 structural and 40 nonstructural, comprising 62.9% of the coding capacity of Yaba virus DNA were detected. These proteins may be further categorized in two classes: early, synthesized in the presence of DNA inhibitor, Ara-c; and late, beginning at 6 hr pi with the bulk of viral protein synthesis occurring later than 30 hr pi The rate of synthesis of viral proteins fell into two patterns: Group A, in which the rate of synthesis decreased; and, Group B, in which the rate of synthesis increased after the initial appearance of the protein. (Author abstract) (35 Refs)

VIRUS ASSOCIATED CELL TRANSFORMATION AND HOST ALTERATION

9. RESCUE OF SV40 FOLLOWING TRANSFECTION OF TC7 CELLS WITH CELLULAR DNAS CONTAINING COMPLETE AND PARTIAL SV40 GENOMES.

Lipotich G, Moyer MP, Moyer RC
Thorman Cancer Res. Lab., Trinity Univ., San Antonio, TX, 78284
MGG; 186(1):78-81 1982

Infectious SV40 virions could be rescued from permissive TC7 cells within one to three subcultures following cotransfection with two cellular DNAs, each containing a complementary portion of the SV40 genome. SV40 virions could also be rescued by transfection of TC7 cells with cellular DNAs from a variety of SV40 transformed cells containing complete genome equivalents but not from cells containing subgenomes alone or defective genomes. Infectious virus was not rescued if the transfecting DNA species was treated with DNAase or if the DEAE-dextran pretreatment of the recipient cells was omitted. (Author abstract) (16 Refs)

10. QUANTITATION OF A 55K CELLULAR PROTEIN: SIMILAR AMOUNT AND INSTABILILTY IN NORMAL AND MALIGNANT MOUSE CELL LINES.

Mora PT, Chandrasekaran K, Hoffman JC, McFarland VW
Macromolecular Biology Section, NCI, NIH, Bethesda, MD, 20205
Mol Cell Biol; 2(7):763-771 1982

Quantitative expression of a specific 55,000 mol wt (55K) cell protein was studied in two groups of mouse fibroblast (clonal) cells from two parental clones, one with high (HT), the other with low tumorigenicity (LT). From the LT clone, tumor lines and clones were obtained by selecting rare, spontaneously transformed highly tumorigenic (mutant) cells. Cells were labeled during exponential growth with 35S-methionine, and the 55K protein was immunoprecipitated with a monoclonal antibody and quantitated. There were low and approx equal amounts of 55K in cells (clones) with both low and high tumorigenicity from both groups of cells; there was no correlation between quantitative expression of 55K protein and cellular tumorigenicity. As shown previously, there was approx 10 to 20-fold more 55K protein in all simian virus 40 (SV40)-transformed T antigen-positive derivative clones. The T antigen-negative revertant tumor lines and clones obtained by an immunological in vivo selection method had low amounts of 55K protein, as in the parent cell before SV40 transformation. In all T antigen negative cells (including highly tumorigenic cells), degradation of the 55K protein was rapid, with a half-life of 15-60 min. The 55K protein was stable in all T antigen-positive cells (half-life greater than 10 hr). There was a very low, or an undetectable amount of the 55K protein in primary cells established from the tumors induced by highly tumorigenic cells. In contrast, primary cells obtained from early murine embryos had large amounts of (stable) 55K proteins. (30 Refs)

11. ASSOCIATION OF SV40 LARGE TUMOR ANTIGEN AND CELLULAR PROTEINS ON THE SURFACE OF SV40-TRANSFORMED MOUSE CELLS.

Santos M, Butel JS
(c/o Butel) Dept. Virology and Epidemiology, Baylor Coll. Medicine, Houston, TX, 77030
Virology; 120(1):1-17 1982

A cellular protein with a mol wt of about 53,000 (53K) and histocompatibility antigens (mouse H-2 antigens) have been reported to be associated with viral-specified proteins in transformed cells. We investigated whether such associations could be detected on the surface of SV40-transformed mouse cells. A differential immunoprecipitation technique was adapted so that surface-associated antigens could be detected independently from intracellular antigens. The precipitated proteins were eluted and analyzed by SDS-polyacrylamide gel electrophoresis. Reconstruction experiments established that SV40 large T antigens (T-ag) released from the nucleus during the extraction procedure was not combining with free antigen-binding sites on antibodies bound to the cell surface in the external reaction, that nuclear unbound T-ag was not exchanging with bound surface antigen during extraction, and that the surface reaction was not due to nuclear T-ag released from dead cells and non-specifically adsorbed onto the surface of living cells. It appears that SV40 large T-ag and cellular 53K protein are associated on the surface as well as within SV40-transformed mouse cells. In contrast, no detergent-stable complex between T-ag and mouse H-2 antigens was detected on the transformed cells. The possibility that molecular interactions between viral- and cell-coded proteins could be involved in determining some of the observed transformation-related cellular phenotypic changes is discussed. (73 Refs)

12. CHARACTERIZATION OF SV40-TRANSFORMED HUMAN LIVER CELLS BEFORE AND AFTER PASSAGE THROUGH CRISIS.

Buitenwerf J, De Jong W, Van Strien A, Van der Noordaa J
(c/o van der Noordaa) Laboratorium voor de Gezondheidsleer, Universiteit van Amsterdam, Mauritskade 57, 1092 AD Amsterdam, The Netherlands
Intervirology; 17(4):222-227 1982

SV40-transformed human liver cells could be passed through crisis after transfection with various DNAs (SV40, BKV, or salmon sperm). The pre- and post-crisis cells were compared with respect to growth behavior and the state of the viral genome. Comparison of the viral inserts in the pre- and post-crisis cells showed minor changes in the integration pattern of the SV40 DNA after recovery from crisis. (Author abstract) (18 Refs)

13. A HIGHLY PREDICTABLE ANIMAL MODEL OF RETINOBLASTOMA.

Kobayashi M, Mukai N, Solish SP, Pomeroy ME
(c/o Mukai) Library, Eye Res. Inst., 20 Staniford St., Boston, MA, 02114
Acta Neuropathol (Berl); 57(2/3):203-208 1982

A highly predictable animal model in newborn inbred CDF rats is described that consistently provides young animals bearing retinoblastoma-like tumors in the vitreous cavity within 1 mo of tumor cell inoculation. The retinoblastoma-like cell line EXP-5 used was derived from an explant of fresh retinoblastoma-like neoplasms induced by adenovirus 12 (Ad 12) in eyes of syngeneic newborn rats. Within 1 mo after intravitreous inoculation of EXP-5 cells, a clinically recognizable ocular tumor was obtained in all 39 rats injected. Immunofluorescent techniques demonstrated Ad 12-specific tumor antigens in cultured tumor cells. Morphologic studies, including use of the light and electron microscope, indicated the tumor cells closely resembled retinoblastoma, with poorly differentiated intracytoplasmic organelles, solitary cilia with a 9 + 0 tubule pattern, and abnormal nuclear membrane associated with a set of basal bodies. A review is presented of transplantable retinal tumors described to date and compared to tumor cell line EXP-5. (15 Refs)

14. AN EXAMINATION OF ADENOVIRUS TYPE 5 MUTANTS FOR THEIR ABILITY TO INDUCE GROUP C ADENOVIRUS TUMOR-SPECIFIC TRANSPLANTATION ANTIGENICITY IN RATS.

Gallimore PH, Williams J
(c/o Williams) Dept. Biological Sciences, Carnegie-Mellon Univ., Mellon Inst., 4400 Fifth Ave., Pittsburgh, PA, 15213
Virology; 120(1):146-156 1982

The expression of Group C adenovirus tumor-specific transplantation antigenicity (TSTA) has been examined in rats using a variety of Type 5 adenovirus (Ad-5) mutants. Transformation-defective Group I E1A mutants (other than Ad-5 hr1 and hr2), Group II E1B mutants, and N gene mutants, produced significantgly lower TSTA or no TSTA when compared with levels of TSTA induced by Ad-2 and Ad-5 wild-type viruses. Two mutants, Ad-5 sub304 and Ad-5 ts125, neither of which possess lesions within the transforming sequences, induced high levels of TSTA in vivo. (Author abstract) (43 Refs)

15. CLONING OF DNA FRAGMENTS FROM THE LEFT END OF THE ADENOVIRUS TYPE 12 GENOME: TRANSFORMATION BY CLONED EARLY REGION 1.

Byrd PJ, Chia W, Rigby PW, Gallimore PH
Dept. Cancer Studies, Univ. Birmingham Medical Sch., Cancer Res. Campaign Lab., Birmingham B15 2TJ, England
J Gen Virol; 60(part2):279-293 1982

The human adenovirus serotype 12 (Ad-12) EcoRI-C DNA fragment (0 to 16.5 map units) was cloned in the plasmid vector pAT153. This cloned Ad-12 EcoRI-C DNA fragment was subcloned generating recombinant plasmids which contained the Ad-12 SalI-C fragment (0 to 10.3 map units), the Ad-12 HindIII-G fragment (0 to 6.8 map units) and the Ad-12 AccI-H fragment (0 to 4.7 map units). Thus, we constructed recombinant plasmids which contain Ad-12 DNA sequences which represent all or part of the virus transforming gene region. The capacity of the cloned Ad-12 EcoRI-C DNA fragment to transform rat cells in vitro was assessed using the focus assay on primary cultures of rat cells. The specific transforming activity of this recombinant plasmid was in the same range as that found for intact Ad-12 DNA. Transformed foci which were induced by the cloned Ad-12 EcoRI-C DNA fragment were established as cell lines and the presence of AD-12 DNA in these lines was demonstrated using the Southern blotting technique. (Author abstract) (47 Refs)

16. GANGLIOSIDE GM1 SENSITIZES TUMOR CELLS TO GROWTH INHIBITORY GLYCOPEPTIDES.

Kinders RJ, Rintoul DA, Johnson TC
Div. Biology, Kansas State Univ., Manhattan, KS, 66506
Biochem Biophys Res Commun; 107(2):663-669 1982

A glycopeptide prepared from the surfaces of cerebral cortex cells of Swiss albino mice by mild proteolysis inhibits cellular protein synthesis in normal but not transformed cells.

Treatment of C3H mouse fibrosarcoma 1316 cells with the glycopeptides resulted in only a limited degree of inhibition of cell protein synthesis compared to polyoma virus-transformed baby hamster kidney cells. The previously described possible role of ganglioside GM1 as a receptor for a number of glycoproteins with physiological activity suggested that the insensitivity of 1316 cells to the glycopeptide growth inhibitor (GGI) might be due to insufficient levels of GM1 in the cell surface membrane. When 1316 cells were incubated with GM1 under conditions in which the ganglioside is incorporated into the cells, the cells became sensitive to GGI. In parallel studies with ceramide, there was no change in 1316 sensitivity. When 1316 cells were incubated with GM1 for 18 hr, they appeared to metabolize GM1 to simpler gangliosides and lost their sensitivity to the glycopeptide inhibitor. (17 Refs)

17. INTEGRATED SHOPE VIRUS DNA IS PRESENT AND TRANSCRIBED IN THE TRANSPLANTABLE RABBIT TUMOUR VX-7.

McVay P, Fretz M, Wettstein F, Stevens J, Ito Y
Dept. Microbiology and Immunology, Univ. California Los Angeles Sch. Medicine, Los Angeles, CA, 90024
J Gen Virol; 60(part2):271-278 1982

To facilitate a molecular analysis of Shope papilloma virus-induced neoplastic cells, we have established a cell line from Vx-7, a transplantable tumor originally induced by the Shope virus. Single phase molecular hybridization and Southern transfer methods were employed to assess copy number and physical state of the DNA, and the extent of transcription. Both tumor and cell line were found to contain multiple copies of the virus genome and these were all integrated into the host cell DNA. Transcripts corresponding to a complexity of approx 1% of the virus genome were detected at low abundance. These results are discussed relative to our earlier findings with tumors induced directly by virus, and to requirements for maintenance of the Vx-7 tumor over the 30 years that it has been in existence. (Author abstract) (22 Refs)

18. CLONING AND STRUCTURAL ANALYSIS OF INTEGRATED WOODCHUCK HEPATITIS VIRUS SEQUENCES FROM HEPATOCELLULAR CARCINOMAS OF WOODCHUCKS.

Ogston CW, Jonak GJ, Rogler CE, Astrin SM, Summers J
Inst. Cancer Res., Fox Chase Cancer Center, Philadelphia, PA, 19111
Cell; 29(2):385-394 1982

Woodchuck hepatitis virus (WHV), like the related hepatitis B virus, induces in its natural host hepatocellular carcinomas that contain integrated viral sequences. As a first step in determining whether and how the integrated sequences contribute to formation of the tumors in which they are found, we have cloned two such integrations of WHV and have determined their structure by restriction mapping and heteroduplex electron microscopy. The identity of the cloned sequences was confirmed by comparison of restriction sites in the clones with those located by Southern blot analysis of tumor DNA. Viral sequences in both integrations are extensively rearranged, and in neither were all parts of the viral genome represented. In this respect, the behavior of WHV in vivo is similar to that of other DNA tumor viruses that have been studied in vitro. We discuss the implications of these results in relation to possible mechanisms for tumor induction by WHV. (Author abstract) (38 Refs)

VIRUS REPLICATION AND EXPRESSION

19. DELETION MUTANTS WHICH AFFECT THE NUCLEASE-SENSITIVE SITE IN SIMIAN VIRUS 40 CHROMATIN.

Gerard RD, Woodworth-Gutai M, Scott WA
Dept. Biochemistry, Univ. Miami Sch. Medicine, Miami, FL, 33101
Mol Cell Biol; 2(7):782-788 1982

Nuclear extracts from in(Or)-1411-infected cells were incubated with MnCl2, allowing endogenous nuclease to cleave within the nuclease-sensitive regions. Introduction of deletions into a simian virus 40 (SV40) variant enabled a quantitative comparison between the nuclease sensitivity of the segment carrying the deletion and the unaltered copy elsewhere on the viral genome. Restriction enzyme analysis and heteroduplex mapping gave patterns identical to that of in(Or)-1411 DNA except for deletion of sequences within the inserted segment. Endonuclease which is endogenous to BSC-1 cell nuclei was used to cleave 32P-labeled chromatin to a limited extent. Variant in(Or)-1411 preferentially cut within each of the duplicate segments. Deletion mutant chromatin displayed nuclease sensitivity in segment I which is comparable to the parent. In(Or)-1411-dl12, -dl14, and -dl46 showed little or no preferential cleavage in region II. Mutants dl9, dl10, dl16, and dl42 retained the characteristic nuclease sensitivity in region II. Locations of the preferentially nuclease-sensitive regions were confirmed by restriction enzyme mapping of the linear DNA with KpnI. Specific cutting frequencies (corrected for a background assumed to be due to random cleavage) obtained for segments I and II in the mutants are presented. (29 Refs)

20. A SMALL SUBCLASS OF SV40 T ANTIGEN BINDS TO THE VIRAL ORIGIN OF REPLICATION.

Scheller A, Covey L, Barnet B, Prives C
Dept. Biological Sciences, Columbia Univ., New York, NY, 10027
Cell; 29(2):375-383 1982

We examined the affinities of SV40 large T antigen for unique viral DNA sequences by binding SV40 Bst NI DNA fragments in extracts of infected or transformed cells, and then immunoprecipitating the T antigen-DNA complex. The G fragment, which spans the viral origin of replication (ori) was quantitatively bound to T antigen. A T-antigen-specific monoclonal antibody (Mcl 7), which recognized only 5%-10% of the T antigen from infected or transformed cells, immunoprecipitated the majority of the ori-binding activity. This suggests that only a minor subclass of wild-type T antigen is active in binding to the origin. C6 cells contain a replication-defective mutant T antigen that when tested in the DNA-binding immunoassay, showed no affinity for the ori fragment. Mcl 7 not only failed to immunoprecipitate ori binding in C6 cells, but also did not detect any labeled C6 T antigen whatever. Thus Mcl 7 recognizes an immunologically distinct subset of wild-type T antigen that comprises the origin-binding form of the viral protein, which is absent in the C6 T antigen population. Mcl 122, which recognizes a 53 kilodalton host protein that complexes with T antigen, immunoprecipitated ori-binding activity from extracts of infected or transformed cells, but not from C6 cells. Thus wild-type T antigen can bind ori sequences even when complexed to the host protein. These data suggest that T antigen consists of different subpopulations with different functions. (Author abstract) (48 Refs)

21. CV-1 CELLS RELEASE PROTEINS THAT FACILITATE INFECTION BY SIMIAN VIRUS 40 AND ECHOVIRUS 6.

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Dept. Immunology and Microbiology, Wayne State Univ. Sch. Medicine, Detroit, MI, 48201
Arch Virol; 72(1/2):55-66 1982

Uninfected simian cells (CV-1) produce two different proteins, one of which enhances the infectivity of echovirus 6 and the other enhances the infectivity of SV40 in less susceptible cells. The enhancers are released by metabolizing cells into the culture medium. The SV40 enhancer protein is larger and less acidic than the echovirus enhancer. The SV40 enhancer protein can be dissociated into two subunits with apparent mol wt of 42,000 and 24,000. The echovirus enhancer protein consists of two subunits with apparent mol wt of 28,000 and 31,000. Electrophoretically purified enhancer proteins interact with virions and retain their biological activities and viral specificities. The SV40 enhancer stimulates expression of SV 40-T antigen under conditions in which untreated virus does not initiate expression of T antigen. This result and the observation that the enhancers do not increase the infectivity of the nucleic acids of their respective viruses suggest that the enhancers act either at the stage of penetration or uncoating. (Author abstract) (13 Refs)

22. DNA SEQUENCES REQUIRED FOR SPECIFIC AND EFFICIENT INITIATION OF TRANSCRIPTION AT THE POLYOMA VIRUS EARLY PROMOTER.

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Transcription and Nucleic Acid Chemistry Laboratories, Imperial Cancer Res. Fund, Lincoln's Inn Fields, London WC2A 3PX, England
Mol Cell Biol; 2(7):737-751 1982

The 5'-flanking sequences of the principle polyoma virus (Py) early promoter region was studied by the construction of deletion and substitution mutants. In vivo effects of certain mutations were measured by assay of the ability of DNAs to transform rat fibroblasts; effects of all mutations on the position and efficiency of in vitro initiation were measured with quantitative assays. A convenient internal control was the secondary Py early promoter region. Sequence requirements for efficient in vivo expression were different from those in vitro. DNA deletions between 200 and 400 base pairs (bp) before the principal cap sites severely inhibited in vivo expression measured as transformation ability, while in vitro transcription was not affected. Viable deletion mutants lacking the principal cap sites and the 'TATA' box were poor templates for in vitro transcription. In vitro analysis of other deletion mutants showed that specific sequences more than 46 bp before the cap sites were important. TATA box removal reduced in vitro transcriptional efficiency but did not alter initiation sites. Synthesis of transcripts with abnormal 5' termini did not occur in vitro until the sequence between the TATA box and the normal cap sites was also deleted. There was a nonspecific requirement for 90-100 bp of DNA 5' to the cap site for optimal transcription of DNA fragments in vitro. (73 Refs)

23. SELECTIVE INHIBITION OF ADENOVIRUS TYPE 2 EARLY REGION II AND III TRANSCRIPTION BY AN ANISOMYCIN BLOCK OF PROTEIN SYNTHESIS.

Shaw AR, Ziff EB
Rockfeller Univ., New York, NY, 10021
Mol Cell Biol; 2(7):789-799 1982

Transcriptional regulation of adenovirus Type 2 (Ad-2) early gene expression was studied. The early period was dissected into a series of stages by blocking protein synthesis with the antibiotic anisomycin. Selective blockage of EII and EIII was used to assess the role of chromatin structure change in the activities on EII and EIII. Anisomycin (100 uM) was added to one portion of an exponentially growing HeLa cell culture, and 30 min later the culture and drug-free control were concentrated for virus infection. In the control culture, EIa, EII, EIII, and EIV were transcriptionally active, and nuclear RNA from each of the regions accumulated. In the drug-treated culture, EIa and EIV were also active, and RNA accumulation was comparable to that of the control. Accumulation of messenger RNA (mRNA) from regions EIa and EIV proceeded at essentially the same rate in the presence or absence of the drug. Anisomycin treatment caused a dramatic decrease, estimated to be greater than 10-fold, in the steady-state level of EII and EIII mRNA. The EII-EIII transcription rate was greatly repressed in the presence of anisomycin. Resultes confirmed that the principal effect of anisomycin is at the level of transcription and is confined to regions EII and EIII. Similarity in the banding patterns suggested that a protein synthesis block which decreases transcription from the EII and EIII promoters by greater than 90% does not cause a difference in the pattern of deoxyribonuclease I sensitivity of the repressed chromatin relative to the transcriptionally active chromatin in control cells. (26 Refs)

24. INDUCTION OF THE SYNTHESIS OF A 70,000 DALTON MAMMALIAN HEAT SHOCK PROTEIN BY THE ADENOVIRUS E1A GENE PRODUCT.

Nevins JR
Rockefeller Univ., New York, NY, 10021
Cell; 29(3):913-919 1982

We have attempted to determine whether any cellular genes are activated as a result of the action of the adenoviral E1A gene. The proteins synthesized in uninfected HeLa cells have been compared to those produced in early adenovirus infected cells. At least one protein, absent from uninfected HeLa cells, was synthesized in large amounts following adenovirus infection. This 70 kd protein was not synthesized in cells infected with the E1A mutant dl312, even when the multiplicity of infection with the mutant was such that the only viral gene not expressed was the E1A gene. Thus the induction of the 70 kd protein requires the expression of the viral E1A gene. The 70 kd protein was also induced by heat shock in uninfected cells. The same 70 kd protein is synthesized in 293 cells, a line of human embryonic kidney cells transformed by a fragment of adenovirus DNA. These cells constitutively express the E1A and E1B genes. (Author abstract) (22 Refs)

25. HERPES VIRUS SPECIFIC ENZYMES: PROPERTIES, PHYSIOLOGICAL ROLES, AND PHARMACOLOGICAL IMPLICATIONS.

Cheng YC, Nakayama K, Derse D, Bastow K, Ruth J, Tan RS, Dutschman G, Caradonna SJ, Grill S
Dept. Pharmacology, Sch. Medicine, Univ. North Carolina, Faculty Lab. Office Building 231-H, Chapel Hill, NC, 27514
Excerpta Med Int Congr Ser; 571:47-56 1982

After infection of cells with various types of herpes virus, a number of new polypeptides are synthesized, a few of which exhibit specific enzyme activity. A number of enzymes (thymidine kinase, DNA polymerase, DNase, ribonucleotide reductase, deoxyuridine triphosphatase, and uracil-DNA-glycosylase) are discussed in relation with their role of beeing potential targets for selective antiviral compounds. (79 Refs)

26. TRANSFECTION WITH THE ISOLATED HERPES SIMPLEX VIRUS THYMIDINE KINASE GENES. II. EVIDENCE FOR AMPLIFICATION OF VIRAL AND ADJACENT CELLULAR DNA SEQUENCES.

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J Gen Virol; 60(part2):209-224 1982

We have previously described several series of biochemical transformation experiments in which small defined portions of herpes simplex virus (HSV-1 and HSV-2) DNA encompassing the thymidine kinase (TK) gene were introduced into Ltk- cells by the calcium transfection procedure. The presence of authentic virus TK enzyme in several subcloned cell lines derived from these experiments was confirmed by either the specific incorporation of [125I]iododeoxycytidine into their nuclei or the inhibition of cell growth by the antiviral drug arabinosyl thymine. A panel of 24 independent Ltk+ cell lines receiving either isolated virus DNA fragments or cleaved plasmid DNAs was examined by blot hybridization for both the presence and copy number of virus TK DNA sequences. Most cell lines contained a single virus DNA fragment covalently joined to host (or carrier Ltk(-)) mouse DNA sequences, but several contained multiple copies of the TK gene. Examination of the structural arrangement of the virus DNA in two early passage multicopy cell lines indicated that the TK gene had integrated into Ltk- cell DNA and then subsequently both viral and flanking cellular sequences were amplified to create up to 20 tandem duplications. In one case, mapping of the adjacent cellular sequences has revealed that the total repeat unit is greater than 23 kilobases (kb) in size. On subsequent passaging, even in HAT medium, the amplified repeat units were not stable and fell to only three to four copies per haploid cell genome. These cell lines should prove useful for additional studies to examine the expression of co-selected non-TK virus sequences and the influence of adjacent cellular DNA sequences on transcription and retransfection of the resident TK gene. (Author abstract) (45 Refs)

27. EXPERIMENTAL STUDIES ON INDUCTION OF CERVICAL CARCINOMA IN MICE BY GENITAL HERPES SIMPLEX VIRUS.

Chen MH, Swen Y

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Excerpta Med Int Congr Ser; 571:358-364 1982

Incidence of tumors in 2-mo-old virgin female hybrid mice was significantly greater in groups that received multiple intravaginal instillations of herpes simplex virus (HSV) than after single such instillations. Single instillations were given 136 mice in Group 1. In Group 2, 93 mice received intravaginal instillations of virus every 2 wk for 4 or 8 times during the first 4 mo. The 81 control mice in Group 3 were treated similarly to those in Group 2, with substitution of saline for HSV. Incidence of cancer was 5/136 (3.67%), 7/93 (7.53%), and 0/0, in Group 1, 2 and 3, respectively. Tumors in Group 1 included squamous cell carcinoma (SCC) of the vagina of the cervix. In Group 2, cancers included adenoid carcinomas of the vagina and cervix, and SCC of the vagina and cervix. A number of precancerous lesions were also found in the experimental mice. All of the cancers found in the study were early infiltrative lesions only detected by microscopic examination at necropsy. (15 Refs)

28. PRELIMINARY STUDY ON THE ROLE OF CO-FACTORS IN INDUCING CERVICAL CANCER IN MICE BY GENITAL HERPES SIMPLEX VIRUS.

Chen MH, Swen Y

Dept. Cell Biology, Inst. Virology, Hupeh Medical Coll., Wuhan City, People's Republic of China

Excerpta Med Int Congr Ser; 571:351-357 1982

The possible interactions of various factors such genital herpes simplex virus (HSV), lymphatic leukemic virus (LLV), HSV and local trauma (LT), and HSV and cortisone, in development of cervical cancer was studied in 2-mo-old random bred female mice. LT was produced by stroking a brush against the cervix. One group of mice received ip cortisone (0.3 mg, 1x/wk) for 3 wk before inoculation with HSV. The study involved the use of 50 controls and 285 surviving mice in six treatment groups. Mice which survived over 1 yr were killed. The entire reproductive tract was studied by permanent histologic preparations. No precancer or cancer was noted in groups treated with HSV (50 mice) or LLV (21 mice) alone. One precancer was noted among 63 mice subjected only to LT. Precancers and cancers were noted, respectively, as follows: in 6 and 3 in 41 mice with HSV + LLV; in 8 and 3 in 68 mice with HSV + LT; and in 6 and 2 in 51 mice with HSV + cortisone. The cancers found included squamous cell carcinoma of the vagina, adenoid carcinoma found both in the cervix and vagina, adenoid carcinomas of the cervix and vaginal fornix, and squamous cell carcinoma in the vaginal fornix. The data suggest possible interaction of HSV and LLV, HSV and LT, and HSV and cortisone in the development of cervical cancer in mice. (15 Refs)

29. INDUCTION OF A TRANSFORMING AGENT(S) BY ABORTIVE INFECTION OF HERPES SIMPLEX VIRUS IN THE CELLS CARRYING ENDOGENOUS SARCOMA VIRUS.

Shimizu Y, Kumagai K

Dept. Microbiology, Tohoku Univ. Sch. Dentistry, Sendai, Japan

Excerpta Med Int Congr Ser; 571:29-33 1982

Abortive infection of herpes simplex virus Type 2 (HSV-2) in the murine and hamster cell lines carrying endogenous sarcoma virus induces an agent(s) with in vitro transforming activity in the culture fluids. Two transformed lines positive for murine sarcoma virus but negative for murine leukemia virus were used: D-56 cells derived from murine cell line NIH 3T3, and HT-1 cells derived from a hamster tumor. Normal NIH 3T3 and baby hamster kidney cells and the transformed cells were infected with HSV-2. No increase of virus progeny was seen with either D-56 or HT-1 cells. After cultivation at 37 C for 1 day, the culture fluids were examined for transforming activity using 3T3 or mouse embryo cells which had been treated with diethylaminoethanol-dextran. The exposed cells were subcultured and tested for the appearance of transformed foci in soft agar medium. After 3-4 wk, 2-3 transformed foci/dish appeared only in the 3T3 cell cultures treated with the culture fluids of infected D-56 cells. No foci could be seen with either infected or uninfected 3T3 cells. These results indicate the HSV-2 was not able to replicate in D-56 cells, but did induce an agent(s) with transforming activity in the culture fluids. The possible nature of the agent(s) is discussed. (9 Refs)

IMMUNOLOGICAL STUDIES

30. DETECTION OF POLYOMA-VIRUS-INDUCED TUMOR ANTIGEN(S) BY MACROPHAGE MIGRATION INHIBITION.

Szigeti R, Dalianis T, Klein G, Ito Y, Magnusson G
Dept. Tumor Biology, Karolinska Inst., S 104 01 Stockholm, Sweden
Int J Cancer; 30(1):69-74 1982

We have applied the macrophage migration inhibition assay to study cell-mediated immune responses to antigens of polyoma-virus-transformed cells. Macrophages from polyoma-virus-immunized mice, but not from control mice, showed significant migration inhibition when exposed to extracts of polyoma-virus-induced mouse and rat transformed cells. Extracts of non-polyoma-induced tumors had no such effect. (Author abstract) (31 Refs)

31. STUDIES ON THE INTERRELATION OF RESISTANCE AND IMMUNITY IN A MOUSE MODEL SYSTEM OF HERPES-SIMPLEX TYPE 2 INFECTION.

Armerding D
Immunology Dept., Sandoz Forschungsinstitut GmbH, Brunner Strasse 59, 1235 Vienna, Austria
Immunobiology; 161(5):415-428 1982

Intraperitoneal (ip) vaccination of mice with attenuated herpes simplex virus Type 2 (HSV 2) induced solid protection to ip infection with pathogenic virus within 2 days. Protection was non-virus-specific until Day 4 after sensitization but increased in specificity thereafter. Normal mice could be protected by adoptively transferred spleen cells, serum, and peritoneal fluid from donors vaccinated seven days before. Virus-specific effector cells induced in the spleen by in vivo ip sensitization with either live, pathogenic, or attenuated virus and tested in a cytotoxicity assay were exclusively B lymphocytes. No functional B cells, but natural killer (NK) cells, could be detected in the unseparated peritoneal exudate cell (PEC) population. Ability to generate HSV2 specific antibody responses did not correlate with natural resistance. (Author abstract) (40 Refs)

32. ROLE OF INTERFERON IN NATURAL KILL OF HSV-1-INFECTED FIBROBLASTS.

Fitzgerald PA, Von Wussow P, Lopez C
Sloan-Kettering Inst. Cancer Res., 425 E. 68th St., New York, NY, 10021
J Immunol; 129(2):819-823 1982

The production of interferon during natural killer (NK) assays against HSV-1-infected fibroblasts (NK(HSV-1)) was studied to determine whether this interferon was responsible for inducing the preferential lysis of herpes-virus-infected target cells over uninfected target cells. The interferon produced during NK(HSV-1) assays was analyzed and found to have the properties of HU-IFN-alpha. Little or no IFN was produced during NK assays against uninfected fibroblasts (NK(FS)) or K562 (NK(K562)) cells. Although the appearance of interferon in the culture supernatants seemed to parallel the development of cytotoxicity during NK(HSV-1) assays, the levels of cytotoxicity and IFN generated did not correlate, arguing against a strict quantitative dependence of cytotoxicity upon IFN production. NK(K562) and NK(FS) cytotoxicity developed with little or no production of IFN. When IFN-pretreated effector cells were used, there was still a preferential lysis of infected over uninfected target cells. This preferential lysis by IFN-treated effector cells of infected over uninfected targets was seen as early as 2 hr into the assay. Anti-IFN antibodies added to the NK assays, although neutralizing all the IFN produced during the assays, had no effect on NK(FS) or NK(K562) cytotoxic activity and caused a slight reduction of NK(HSV-1) activity only in one of three experiments. We conclude that although IFN is generated during NK(HSV-1) assays, this IFN cannot solely account for the increased lysis of infected over uninfected cells and that NK(HSV-1) activity is in some other way dependent on the virus infection. (Author abstract) (34 Refs)

33. SPECIFIC TUMOR IMMUNITY PRODUCED BY THE INJECTION OF VACCINIA VIRAL ONCOLYSATES.

Wallack MK
Dept. Surgery, Washington Univ. Sch. Medicine, St. Louis, MO, 63110
J Surg Res; 33(1):11-16 1982

A live vaccinia vaccine virus-augmented and lysed tumor cell vaccine (vaccinia oncolysate) is used as a specific active immune mechanism stimulator in a syngeneic SV40-transformed BALB/c male mouse peritoneal macrophage tumor system. BALB/c mice pretreated with sc injections of vaccinia oncolysates prior to challenge with tumor cells failed to develop tumors at the injection sites. Cross-immunization and immunotherapy experiments were also performed. Because of the encouraging results in this murine tumor system, preliminary trials were instituted to evaluate vaccinia oncolysates as an immunotherapeutic agent in human cancer. (Author abstract) (15 Refs)

OTHER RELATED STUDIES

34. POLYOMA VIRUS MUTANTS AS PROBES OF VARIETY AMONG MOUSE EMBRYONAL CARCINOMA CELL LINES.

Georges E, Vasseur M, Blangy D
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Differentiation; 22(1):62-65 1982

Embryonal carcinoma (EC) cells are resistant to infection with polyoma virus and become permissive when allowed to differentiate. Polyoma host-range (PyEC) mutants have been selected on two embryonal carcinoma cell lines, PCC4 and F9, which differ in their differentiation potential, both in vitro and in vivo. Since PyEC mutants selected on one line failed to develop, or developed only poorly on the other, we used these two classes of mutants as probes towards several EC lines which differed in their origin and differentiation properties. From their susceptibility to either mutant, and from the effect of temperature upon the efficiency of infection, we inferred a classification of these teratocarcinoma cell lines, which is in agreement with a previous one based upon metabolic coupling. We also discuss results indicating that different EC cell lines might represent steps in the process of cell determination at the embryonic level. (Author abstract) (26 Refs)

35. HERPESVIRUSES IN METASTATIC LUCKE RENAL ADENOCARCINOMA.

McKinnell RG, Cunningham WP
Nuffield Dept. Pathology, John Radcliffe Hosp., Headington, Oxford OX3 9DU, England
Differentiation; 22(1):41-46 1982

The etiologic agent of the renal adenocarcinoma of leopard frogs, Rana pipiens, is the Lucke tumor herpesvirus (LTHV). The virus is easily detected with thin section electron microscopy in primary tumors of frogs which have been exposed to a cold environment. Several spontaneous metastatic nodules and a large primary tumor were detected at autopsy of a frog which had been maintained at 4 C for 73 days. LTHV was found not only in the primary tumor, as previously reported, but also was present in metastatic tumor cells in the liver, fat body, and bladder. The presence of LTHV in metastatic cells demonstrates that the differentiated state of primary Lucke tumor cells is retained in its metastatic colonies even at the fine structure level revealed by electron microscopy. (Author abstract) (25 Refs)

36. SEASONAL AND GEOGRAPHICAL CHANGES OF SPONTANEOUS SKIN PAPILLOMAS IN THE JAPANESE NEWT CYNOPS PYRRHOGASTER.

Asashima M, Komazaki S, Satou C, Oinuma T
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Cancer Res; 42(9):3741-3746 1982

The occurrence of spontaneous skin papillomas in Japanese newts (Cynops pyrrhogaster) from Niigata prefecture in Northern Japan was monitored over four seasons covering a period of 2 yr. Of 13,613 newts, 249 were found to possess papillomas. The percentage of newts with papillomas was highest in autumn, ranging from 1.93 to 5.45% of the total av. These values were more than four times as high as values obtained in the other three seasons (0.16 to 0.50%). A total of 12,167 newts were collected from their natural environment in 10 prefectures of Japan in the autumn of 1980 and 1981. Newts collected from the northern, seaside prefectures had higher papilloma rates (1.00 to 5.45%) than did newts from the southern, Pacific Ocean prefectures of Japan (0 to 0.27%). Male and female newts were affected equally often by the epitheliomas. Virus-like bodies, resembling herpes-type virus, were found in the cytoplasm of the epitheliomas. These virus-like bodies were not seen in the control epithelium of newts with normal skin and without papilloma or in nonaffected regions of newts with papilloma. It is suggested that tumor and virus are causally related. (Author abstract) (30 Refs)

37. SPECTRUM OF SENSITIVITY TO ACYCLOVIR OF HERPES SIMPLEX VIRUS CLINICAL ISOLATES.

McLaren C, Sibrack CD, Barry DW
Infectious Diseases Lab., Burroughs Wellcome Co., 3030 Cornwallis Road, Research Triangle Park, NC, 27709
Am J Med; 73(1A):376-379 1982

HSV-1 and HSV-2 clinical isolates were tested for their in vitro sensitivity to acyclovir. The median ID50 for 32 genital HSV-2 isolates was 0.215 ug/ml and this was not significantly higher than a median value of 0.125 ug/ml for 28 oral HSV-1 isolates ($p = 0.08$). A wider range of ID50S was observed with the HSV-2 isolates compared with the HSV-1 isolates. The concentration of drug required to inhibit virus replication by 90 and 99% was approximately 10-and 100-fold greater than that producing 50% inhibition. (Author abstract) (12 Refs)

38. DISEASE AND LATENCY CHARACTERISTICS OF CLINICAL HERPES SIMPLEX VIRUS ISOLATES AFTER ACYCLOVIR THERAPY.

Sibrack CD, McLaren C, Barry DW
(c/o Barry), Wellcome Res. Lab., Research Triangle Park, NC, 27709
Am J Med; 73(1A):372-375 1982

Herpes simplex virus (HSV) Type 1 from a bone marrow transplant recipient and HSV Type 2 from a patient with genital herpes infection were examined for sensitivity to acyclovir after both patients received therapy with the drug. A 38-and 83-fold shift in sensitivity was detected in association with a marked decrease in viral thymidine kinase activity in isolates from both patients. The resistant HSV-1 isolate was approximately 900x less neurovirulent for BALB/c mice but had similar cutaneous virulence in hairless mice compared with the patient's sensitive strain. In contrast, there was no difference in pathogenicity between the sensitive and resistant HSV-2 isolates. Latency was detected in the trigeminal ganglia of mice after snout inoculation with both the sensitive and resistant HSV-1 isolates. The ganglion isolate from the resistant HSV-inoculated mouse was found to be sensitive to acyclovir, implying a selection for or reversion to the sensitive phenotype. No trigeminal ganglion latency was detected after inoculation with either HSV-2 isolate. Resistance to acyclovir can arise during therapy as a results of diminished viral thymidine kinase activity but does not appear to be associated with increased virulence. (Author abstract) (20 Refs)

39. GENETIC MECHANISMS OF RESISTANCE OF ACYCLOVIR IN HERPES SIMPLEX VIRUS.

Crumpacker CS, Schnipper LE, Chartrand P, Knopf KW
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Am J Med; 73(1A):361-368 1982

Resistance to acyclovir in herpes simplex virus (HSV) is mediated by two genetic loci on the herpes virus genome: viral thymidine kinase and DNA polymerase. Passage of HSV in the presence of 10 uM or 2 uM acyclovir readily coselects for virus deficient in the expression of viral thymidine kinase and resistance to acyclovir. Other workers have shown that passage of HSV in serum-starved cells in the presence of acyclovir selects for a mutant virus possessing a thymidine kinase with an altered substrate specificity and inability to phosphorylate acyclovir. Mutants with temperature-sensitive lesions in the viral DNA polymerase loci of HSV also exhibit resistance to antiviral drug phosphonoacetic acid (PAA) and acyclovir. The mutations conferring resistance to PAA and acyclovir are closely linked within the DNA polymerase locus. The map limits containing mutations that confer resistance to PAA and acyclovir are found within a 1.3 kilobase pair (kbp) region of DNA sequences in HSV-2 (map unit 40.2 to 41.0) and within a 2.6 kbp region of DNA sequence in HSV-1 (map units 40.2 to 41.8). The DNA polymerase loci of HSV also contains a region of DNA sequences to the left of the region defining the resistance mutation which determines the type specific sensitivity of HSV-1 and HSV-2 to acyclovir. Two HSV-1/HSV-2 recombinants were selected for further analysis of resistance. Viral DNA polymerase enzymes were purified from the R6-26 and R6-34 recombinants; the resistant recombinant R6-34 was shown to process an altered viral DNA polymerase. This altered viral DNA polymerase exhibited resistance to three inhibitors of DNA polymerase activity, PAA, acyclovir-TP, and 2'3'dideoxynucleotide triphosphate (didNTPs). The polymerase from R6-34 and the resistant parent possess a much higher Ki for the PAA and the didNTP than do the sensitive strains. A

1.3 kbp region of HSV-1 DNA within the DNA polymerase locus contains resistance mutations to three DNA polymerase inhibitors, codes for an amino acid sequence of 42,000 mol wt, and defines an active center of the HSV DNA polymerase enzymes. (Author abstract) (32 Refs)

40. EFFECT OF 9-(2-HYDROXYETHOXYMETHYL)GUANINE ON VIRAL-SPECIFIC POLYPEPTIDE SYNTHESIS IN HUMAN CYTOMEGALOVIRUS-INFECTED CELLS.

Mar EC, Patel PC, Huang ES
Cancer Res. Center, Sch. Medicine, Univ. North Carolina, Chapel Hill, NC, 27514
Am J Med; 73(1A):82-85 1982

Plaque-forming assay resulted in a 50% inhibitory dose by 9-(2-hydroxyethoxymethyl)guanine (acyclovir) against Towne strain human cytomegalovirus (HCMV) of approximately 98 umol. At the drug concentration of 200 umol, we did not detect any significant inhibition of viral DNA synthesis by cRNA-DNA hybridization. However, at this drug concentration, the synthesis of at least two viral-specific late polypeptides (150K and 67K) was significantly retarded up to 48 hr after infection, but resumed at 72 hr. These data suggest that acyclovir or its in vivo transformed derivative had a transient effect on viral-specific polypeptide synthesis in HCMV-infected human fibroblasts at a high drug concentration. (Author abstract) (19 Refs)

41. REPLICATION OF EPSTEIN-BARR VIRUS DNA IN LYMPHOBLASTOID CELLS TREATED FOR EXTENDED PERIODS WITH ACYCLOVIR.

Colby BM, Shaw JE, Datta AK, Pagano JS
Cancer Res. Center, Univ. North Carolina, Box 3, Swing Building, 217H, Chapel Hill, NC, 27514
Am J Med; 73(1A):77-81 1982

An Epstein-Barr virus (EBV)-producing human lymphoblastoid cell line (P3HR-1) was characterized after prolonged exposure to 9-(2-hydroxyethoxymethyl)guanine (acyclovir). P3HR-1 cells grown for 11 mo in the presence of acyclovir had the same number of EBV genomes (approx 15/cell) as did short-term acyclovir-treated cells. After removal of the drug, the number of EBV genome equivalents of long-term acyclovir-treated cultures (15/cell) returned to P3HR-1 control levels (340/cell). Indirect immunofluorescence studies demonstrated reduced percentages of long-term (6 to 12 mo) acyclovir-treated P3HR-1 cells expressing both EBV early antigens (EA) and viral capsid antigens (VCA). This contrasts with short-term acyclovir-treated cultures where EA is expressed at normal levels even though VCA expression is reduced. Restriction fragment patterns of the total EBV DNA from both short- and long-term acyclovir-treated cultures were identical with the exception of two fragments present in short- but not long-term acyclovir-treated cultures. EBV-associated DNA polymerase was present in long-term acyclovir-treated cultures with the level of activity, as determined by assay in vitro, comparable to that in untreated P3HR-1 cells. The polymerase activity in the treated cells was sensitive in vitro to acyclovir triphosphate, N-ethylmaleimide (NEM), and phosphonoacetic acid (PAA). These studies demonstrate that acyclovir inhibits the EBV-productive cycle in P3HR-1 cells continuously during extended periods of treatment. (Author abstract) (21 Refs)

42. EFFECT OF ACYCLOVIR ON HERPES SIMPLEX VIRUS REPLICATION IN A PERSISTENTLY INFECTED HUMAN LYMPHOBLASTOID CELL LINE (P3HR-1).

Shaw JE, Colby BM, Leong J, Bolling TJ
Univ. North Carolina, Box 3, Swing Building, 217H, Chapel Hill, NC, 27514
Am J Med; 73(1A):72-76 1982

We examined the effect of acyclovir on herpes simplex virus (HSV) replication in the persistently infected human lymphoblastoid cell line, P3HR-1. During 10 days of continuous exposure of P3HR-1 cells to 100 uM acyclovir there was a decrease in the number of cells expressing HSV antigens, the number of copies of HSV DNA per cell, and the infectivity of extracellular virus. A significant increase in each of these was detected after 14 days of continuous exposure of P3HR-1 cells to the drug, demonstrating that the replication of HSV has become resistant to inhibition by acyclovir. No apparent resistance of EBV replication to inhibition by acyclovir, however, was detected in HSV-infected P3HR-1 cells. The number of copies per cell of EBV and HSV DNA increased after exposure of HSV-infected P3HR-1 cells to the tumor promoter TPA (12-O-tetradecanoyl-phorbol-13-acetate). When cells were exposed continuously to acyclovir for 4 mo and then exposed to acyclovir and TPA simultaneously, the number of copies per cell of HSV DNA increased, but the number of copies per cell of EBV DNA increased by only a few, if any. The detection of P3HR-1 cultures of individual cells expressing both HSV and EBV antigens suggested that HSV and EBV productive cycles could occur simultaneously within the same cell. However, single cells very rarely expressed both antigens simultaneously. (Author abstract) (19 Refs)

43. ACYCLOVIR INHIBITION OF VIRAL DNA CHAIN ELONGATION IN HERPES SIMPLEX VIRUS-INFECTED CELLS.

McGuirt PV, Furman PA
Dept. Experimental Therapy, Wellcome Res. Lab., Research Triangle Park, NC, 27709
Am J Med; 73(1A):67-71 1982

The effect of acyclovir on DNA synthesized in HSV-1-infected cells was examined. Viral DNA synthesis was significantly reduced in the presence of 10 uM acyclovir monitored by cRNA-DNA hybridization and isopycnic centrifugation in cesium chloride. DNA synthesized in infected cells in the presence of acyclovir appeared to be chain-terminated when evaluated by rate zonal centrifugation in alkaline sucrose. Cellular DNA synthesis in actively replicating uninfected cells in the presence of acyclovir was evaluated by isopycnic centrifugation. Cellular DNA synthesis appeared unaffected at concentrations of acyclovir up to 100 uM. (Author abstract) (7 Refs)

44. DIFFERENT IN VITRO EFFECTS OF DUAL COMBINATIONS OF ANTI-HERPES SIMPLEX VIRUS COMPOUNDS.

Schinazi RF, Nahmias AJ
Dept. Pediatrics, Div. Infectious Diseases, Allergy and Immunology, Emory Univ. Sch. Medicine, 69 Butler St., S.E., Atlanta, GA, 30335
Am J Med; 73(1A):40-48 1982

Five promising antivirals have been tested individually and in pairs on herpes simplex virus (HSV) Types 1 (Strain F) and 2 (Strain G) in Vero cells. These are: 9-(2-hydroxyethoxymethyl)guanine (acyclovir, ACV), 9-beta-D-arabinofuranosyladenine (ara-A), 5-trifluorothymidine (TFT), E-5-(2-bromovinyl)-2'-deoxyuridine (BVDU), and phosphono-

formate (PFA). Various types of interactions depending on virus type were observed: synergistic [ara-A/TFT; ara-A/BVDU (G); BVDU/PFA (F)]; additive [ACV/ara-A; ACV/TFT; ACV/BVDU; ACV/PFA (G); BVDU/TFT; PFA/ara-A; PFA/TFT]; and sub-additive [ACV/PFA (F); ara-A/BVDU (F) and BVDU/PFA (G)]. Neither antagonism nor interference was noted for any combinations. (Author abstract) (35 Refs)

45. PRECLINICAL TOXICOLOGY PROFILE OF ACYCLOVIR: AN OVERVIEW.

Tucker WE
Dept. Toxicology and Experimental Pathology, Wellcome Res. Lab., Research Triangle Park, NC, 27709
Am J Med; 73(1A):27-30 1982

Numerous and extensive toxicologic studies of acyclovir using animal and in vitro assays indicated a low order to toxicity for this antiviral nucleoside analog. Primary cytotoxic effects occurred only at extremely high drug concentrations in plasma or tissue cultures. This correlates well with the high selectivity of the drug for herpes viruses and its low toxicity for uninfected cells in tissue culture. Secondary toxicity in the form of precipitation of acyclovir crystals in the lower nephron of the kidney occurred after rapid intravenous bolus injection causing transient impairment of renal function in the dog and obstructive nephropathy in the rat. These secondary effects were related to the relatively low solubility of acyclovir in the urine. Dermal and ophthalmic formulations were well-tolerated. No significant primary irritation nor systemic toxic manifestations were associated with the application of these materials. A test in guinea pigs gave no indication that acyclovir has sensitization potential. Acyclovir had no effect on reproductive processes or prenatal, perinatal, or postnatal development of offspring in studies with rats, rabbits, and mice. Positive results occurred in 2 of 11 in vitro mutagenicity assays. These involved chromosomal damage at extremely high drug concentrations. In contrast, no evidence of chromosomal damage occurred in three in vivo assays at nontoxic dosage levels and under conditions more relevant to clinical use of the drug. While one of two cell transformation assays gave positive results, no oncogenic potential for acyclovir was indicated by lifetime carcinogenicity bioassays in rats and mice. Numerous in vitro and in vivo assays gave no indication that acyclovir might significantly alter immunologic responses at clinically relevant doses. (Author abstract) (7 Refs)

46. MECHANISM OF ACTION AND SELECTIVITY OF ACYCLOVIR.

Elion GB
Wellcome Res. Lab., 3030 Cornwallis Road, Research Triangle Park, NC, 27709
Am J Med; 73(1A):7-13 1982

Acyclovir, an acyclic purine nucleoside analog, is a highly potent inhibitor of herpes simplex virus (HSV), Types 1 and 2, and varicella zoster virus, and has extremely low toxicity for the normal host cells. This selectivity is due to the ability of these viruses to code for a viral thymidine kinase capable of phosphorylating acyclovir to a monophosphate; this capability is essentially absent in uninfected cells. The acyclovir monophosphate (acyclo-GMP) is subsequently converted to acyclovir triphosphate (acyclo-GTP) by cellular enzymes. Acyclo-GTP persists in HSV-infected cells for many hr after acyclovir is removed from the medium. The amounts of acyclo-GTP formed in HSV-infected cells are 40 to 100x greater than in uninfected Vero cells. Acyclo-GTP acts as a more potent inhibitor of the viral DNA polymerases than of the cellular polymerases. The DNA polymerases of HSV-1 and HSV-2 also use acyclo-GTP as a substrate and incorporate acyclo-GMP into the DNA primer-template to a much greater extent than do the cellular enzymes. The viral DNA polymerase binds strongly to the acyclo-GMP-terminated template, and is thereby inactivated. (Author abstract) (14 Refs)

47. PERSPECTIVES ON INTERACTIONS OF ACYCLOVIR WITH EPSTEIN-BARR AND OTHER HERPES VIRUSES.

Pagano JS, Datta AK
Sch. Medicine, Univ. North Carolina, Chapel Hill, NC, 27514
Am J Med; 73(1A):18-26 1982

Acyclovir [9-(2-hydroxyethoxymethyl)guanine] inhibits Epstein-Barr virus (EBV) replication in lymphoblastoid cells at concentrations nontoxic to cellular growth. The mode of action of the drug against EBV differs from the mechanism described in herpes simplex virus systems. Due to the absence of virus-specified thymidine kinase, the drug is poorly phosphorylated in EBV-infected cells. The extent of monophosphorylation is similar both in mock-infected and EBV-infected cells. Despite weak phosphorylation of the drug, the replication of linear EBV DNA is inhibited due to exquisite sensitivity of the viral DNA polymerase. Activation of acyclovir does not require phosphorylation by virus-specified thymidine kinase. Inhibition of different herpes-group viruses depends on three variable factors: degree of phosphorylation, cellular metabolism of the drug, and degree of sensitivity of the viral polymerase. Interaction of acyclovir-triphosphate with EBV DNA polymerase is reversible. Cells infected with EBV and treated with acyclovir resume virus replication following removal of the drug even after long exposure. Acyclovir inhibits replication of linear genomes and stops production of virus, but has no effect on latent cellular infection. These results lead us to predict that acyclovir will suppress, but not cure, EBV infection. (Author abstract) (33 Refs)

48. HEAT-RESISTANT INFECTIVITY OF HERPES SIMPLEX VIRUS REVEALED BY VIRAL TRANSFECTION.

Fenyves A, Strupp L
Paul Ehrlich Inst., Paul-Ehrlich-Strasse 42-44, 6000 Frankfurt am Main, W. Germany
Intervirology; 17(4):228-239 1982

Following apparently effective inactivation of herpes simplex virus Types 1 and 2 at 56 or 70 C, significant infectivity was found upon viral transfection using the calcium-dimethylsulfoxide technique. Infectivity was quantitated in RSB-1 (rabbit skin fibroblast) and BHK-21 cells, and dose-response curves were found to depend on both the cell system and side effects of heat. Titers were dependent upon thermal treatment, as well as initial infectivity and cell origin of virus, and showed no or only moderate decrease after 6-hour exposures. There was no liberation of DNA or DNA- protein complexes, since infectivity was DNase- and trypsin-DNase-resistant. Upon purification, activity was found associated with particles that had retained their gross physical integrity. Obviously, protein(s) involved in initiation of infection was primarily altered. Heated virus was also insensitive to antiserum, which prevented replication of unheated, transfected virus. (Author abstract) (23 Refs)

49. RECENT AND FUTURE DEVELOPMENTS IN ANTIVIRAL CHEMOTHERAPY.

Prusoff WH, Chen MS, Lee JJ, Lin TS, Mancini WR, Otto MJ, Spivack J

Dept. Pharmacology, Yale Univ. Sch. Medicine, New Haven, CT, 06510

Excerpta Med Int Congr Ser; 571:428-436 1982

Recent developments in clinically effective antiviral drugs are reviewed. Since toxicity in the uninfected cell is a major problem, attention should be given to drugs that are not active in the uninfected cell, but have activity in the infected cell. A discussion is presented of: (I) enzymes that catalyze reactions unique to the virus infected cell, and are also essential for viral replication, and (II) enzymes which are sufficiently different from their host cell counterparts, so that selective intervention may be possible. Further study of the antiviral state produced by interferon may result in important future developments. Other developments looking more into the future may involve selective binding, which evolves from studies which indicate formation and regulation of the herpes simplex virus-proteins alpha, beta, and gamma in a sequential and interdependent manner. Other approaches involve selective hybridization of inhibitory polynucleotides that are design based on the identification of unique sequences of viral messenger-RNA. Other approaches involve use of selective penetration, 'suicidal activation', temperature-sensitive liposomes, and magnetically responsive albumin microspheres for delivery of drugs. The problem of latency must be attacked. The most important development of recent years is the knowledge that antiviral drugs can be formulated based on a qualitative difference created specifically by the virus. (30 Refs)

50. ANTIHERPETIC CHEMOTHERAPY IN CHINA.

Chen HS, Tien PY

Inst. Antibiotics, Tian-Tan Beijing, People's Republic of China

Excerpta Med Int Congr Ser; 571:183-191 1982

A discussion is presented of in vitro, in vivo and clinical studies of various antiherpetic agents: 5-iodo-2'-deoxyuridine (IDU), 1-beta-D-arabinofuranoysylcytosine (ara-C), 9beta-D-arabinofuranosyladenine (ara-A), 2-2'-cyclocytidine (CC), 2-2'cyclocytidine-5'-monophosphate (CCMP), acycloguanosine (ACV), phosphonoacetate, phosphonoformate, polyinosinic acid:polycytidylic acid, and tilorone. In vitro studies were performed in PRK, HEK or human diploid cells against herpes simplex virus (HSV) I and II. Electron microscopic studies are presented of drug-treated HSV-infected cells in tissue culture. In vivo studies are reported on herpes keratitis in rabbits, herpes encephalitis in mice, herpes cutaneous infection in guinea pigs, and herpes vaginitis in mice. In clinical trials with herpes keratitis in a number of hospitals, IDU and ara-A were effective and are now in regular clinical use; ara-C, while effective, showed a toxic effects on the cornea and prolonged healing. Topical 0.5% CCMP in 21 cases of different types of herpes keratitis showed good therapeutic effect compared to CC. Topical ACV shows promise in the treatment of HSV keratitis. Poly I:C treatment of patients with herpes zoster (1-2 mg 2x/wk, sc) led to a significant shortening of disease; there were no severe side effects, except a few cases of transient low grade fever. Recurrence of disease and development of drug resistance are continuing problems. (20 Refs)

51. CHEMOTHERAPY ON HERPES VIRUS, PARTICULARLY THE EFFECT OF ANTIVIRAL AMINO ACID ANALOGUES.

Toyoshima S, Fukuma M, Fujita H, Seto Y

Div. Chemotherapy, Pharmaceutical Inst., Sch. Medicine, Keio Univ, Tokyo, Japan

Excerpta Med Int Congr Ser; 571:148-156 1982

The characteristics and antiviral effects of two selected amino acid analogs, N-naphthalenesulfonyl-DL-tryptophan (A-91) and N-ethyloxycarbonylaminomethyl-L-isoleucine (A-145), are presented. A-91 shows activity in various studies with herpes simplex virus (HSV) Type 1 and 2, and vaccinia virus in vitro and in vivo. A-91 and A-145 during a 1 hr absorption period did not affect the formation of plaques by HSV-1 and HSV-2 and did not inactivate viruses directly. The compounds do not induce interferon in mice. Results indicate both compounds inhibit viral multiplication but not at a very early stage. In female mice intravaginally infected with HSV-2, macroautoradiographic studies after iv administration of 14C-A-92 or 14C-A-145 showed high radioactivity in the genital organs with consequent prolongation of life span. Since HSV-2 is indicated as one of the possible causes of cervical cancer, these compounds may act as antitumor agents on cervical cancer. (5 Refs)

52. CHEMISTRY AND POTENT ANTIVIRAL ACTIVITY OF 2'-FLUORO-5-SUBSTITUTED-ARABINOSYL-PYRIMIDINE NUCLEOSIDES.

Fox JJ, Watanabe KA, Lopez C, Philips FS, Leyland-Jones B

Sloan-Kettering Inst. for Cancer Res., 145 Boston Post Road, Rye, NY, 10580

Excerpta Med Int Congr Ser; 571:135-147 1982

The chemical synthesis and some experimental and clinical studies on the antiviral activity of a number of 2'-fluoro-5-substituted-arabinosyl-pyrimidine nucleosides are presented. 2-Fluoro-5-iodo-ara-cytidine (FIAC) is effective against herpes simplex virus (HSV) Type 1 and 2 and is less toxic than other antiherpes compounds currently in use. This selective activity depends in part on the virus-specified thymidine kinase. While FIAC structurally is an analogue of ara-C (1-beta-arabinofuranosylcytosine), it is not reversed by deoxycytidine. Various in vitro and in vivo studies on FIAC and 2-fluoro-5-methyl-ara-uridine (FMAU) indicate their utility against HSV-1 infections. Clinical studies with FIAC are reviewed. Dose-limiting toxicity is hematopoietic. A randomized double-blind Phase II trial has been initiated to test the antiviral efficacy of FIAC compared to arabinosyladenine in immunosuppressed patients with serious viral infections (herpes zoster virus and HSV). Two other trials have been initiated in a bone marrow population. Early clinical trials with FIAC are most encouraging. (29 Refs)

53. TUMOR VIRUS INDUCTION AND HOST CELL CAPACITY INACTIVATION: POSSIBLE IN VITRO TESTS FOR PHOTOSENSITIZING CHEMICALS.

Bockstahler LE, Coohill TP, Lytle CD, Moore SP, Cantwell JM, Schmidt BJ

Bureau Radiological Health, Public Health Service, Food and Drug Admin., U.S. Dept. Health and Human Services, 5600 Fishers Lane (HFX-120), Rockville, MD, 20857

JNCI; 69(1):183-187 1982

The responses of two in vitro mammalian virus-host cell systems to the photosensitizing chemicals proflavine sulfate and 8-methoxypsoralen (8-MOP) in the presence of light are de-

scribed. Infectious simian virus 40 (SV40) could be induced from SV40-transformed hamster cells by treatment with proflavine plus visible light or 8-MOP plus near UV radiation. The same photosensitizing treatments inactivated the capacity of monkey cells to support the growth of herpes simplex virus. SV40 induction and inactivation of host cell capacity for herpesvirus growth might be useful as screening systems for testing the photosensitizing potential of chemicals. Advantages and disadvantages associated with each system are discussed. (Author abstract) (26 Refs)

54. AUGMENTED IMMUNOGENICITY OF LEWIS LUNG CARCINOMA BY INFECTION WITH HERPES SIMPLEX VIRUS TYPE 2.

Reiss-Gutfreund RJ, Nowotny NR, Dostal V, Wrba H
Inst. Cancer Res., Univ. Vienna, Borschkegasse 8a, A-1090 Vienna, Austria
Eur J Cancer Clin Oncol; 18(6):523-531 1982

In vitro, LLT cells sustain HSV-2 replication without evidence of lysis. Simultaneously, multiplication of the cells is stimulated. These xenogenized cells were tested for their immunopotentiating capacity: three-step immunization with xenogenized viable cells conferred significantly augmented transplantation resistance to a challenge graft with 4 x 10(4) intact LLT cells. Latency periods preceding tumor formation were increased and 15% of the mice failed to form primary tumors. Metastasis was likewise decreased and 25% of the mice had healthy lungs. Immunopotentiation, however, did not suffice to significantly protect against a challenge with 6 x 10(4) intact cells. The presence of virus-specific neoantigens on HSV-2-infected viable cells was demonstrated by the progressively increasing number of rejections of 4 x 10(4) xenogenized cells during the successive immunization steps. Immunization with non-viable LLT cells did not augment resistance to challenge. (Author abstract) (31 Refs)

55. THE BINDING OF SIMIAN VIRUS 40 LARGE T ANTIGEN TO THE POLYPHOSPHATE BACKBONE OF NUCLEIC ACIDS.

Montenarh M, Henning R
Dept. Biochemistry, Ulm Univ., P. O. Box 4066, D-7900 Ulm, W. Germany
Biochim Biophys Acta; 697(3):322-329 1982

Simian virus 40 (SV40) large tumor antigen (T antigen), a phosphoprotein found in nuclei of SV40-infected and -transformed cells, binds nonspecifically to DNA. To study this mechanism the binding properties of T antigen to double-stranded (ds) and single-stranded (ss) DNA-cellulose as well as to phosphocellulose were compared. After incubation of [35S]methionine or [3H]leucine/[32P]phosphate radioactively-labeled cell extracts at different pH values (6.0, 7.3, 9.0) with DNA- or phosphocellulose, bound and unbound species of T antigen were purified and analyzed by SDS-polyacrylamide gel electrophoresis for both the yield and the possible correlation with protein phosphorylation. T antigens bound with comparable affinities to ds- and ss-DNA-cellulose and phosphocellulose. These results suggest the binding of T antigen to the polyphosphate backbone of DNA as a molecular mechanism for its non-specific binding. The evidence for this observation was supported by blocking the binding of T antigen to DNA-cellulose by divalent cations (Ca++, Mg++). 3H/32P ratios of T antigen obtained by double-labeling cells for various times imply that higher phosphorylated forms of T antigen bound more strongly to ds- and ss-DNA as well as to phosphocellulose. Thus, in the presence of cellular proteins and other components the binding activity of T antigen to the polyphosphate backbone of DNA seems to be positively correlated with its phosphorylation. These observations are consistent with the hypothesis that the binding affinities of SV40 T antigen to host cell DNA may be regulated by its phosphorylation. (Author abstract) (24 Refs)

56. ISOLATION AND CHARACTERIZATION OF THE GROWTH-STIMULATING FACTOR IN SUPERNATANTS OF HERPES SIMPLEX VIRUS TYPE 2 INFECTED CELLS.

Reiss-Gutfreund RJ, Letnansky K, Desser H
Inst. Cancer Res., Borschkegasse 8a, A-1090 Vienna, Austria
Oncology; 39(5):319-324 1982

Lytic infection of CV1 cells with herpes simplex virus Type 2 does not stimulate ornithine decarboxylase activity and there is no correlation between polyamines and a growth-stimulating factor (GSF) which is present in the supernatant of the cultures. The factor was partially purified by gel filtration on Sephadex G-50 and polyacrylamide gel electrophoresis. Gel filtration as well as dialysis through membranes with different pore diameters indicated a mol wt between 3,500 and 10,000 daltons. GSF is not extracted from aqueous solutions by diethyl ether. The activity is partially sensitive to trypsin digestion and is completely destroyed by periodate oxidation. These results suggest that GSF is a glycoprotein or a glycopeptide whose mitogenic activity resides mainly in the sugar moiety. (Author abstract) (24 Refs)

57. RESTRICTION ENZYME CLEAVAGE OF ULTRAVIOLET-DAMAGED DNA.

Cleaver JE, Samson L, Thomas GH
Lab. Radiobiology and Environmental Health, Univ. California, San Francisco, CA, 94720
Biochim Biophys Acta; 697(2):255-258 1932

SV40 and pBR322 DNAs damaged by ultraviolet light were cleaved abnormally by several restriction enzymes because of damage to pyrimidines in the recognition sequences. The use of a tandemly duplicated plasmid provided a particularly sensitive target molecule for detecting pyrimidine dimers and other possible photoproducts. The relative efficiency with which cleavage was blocked (HindIII greater than TaqI greater than EcoRI greater than BamI greater than SalI much greater than HhaI,HaeIII) corresponds approximately to the relative frequency of pyrimidine dimer formation in the recognition sequences, but at a slightly higher frequency in potential sites for the non-cyclobutane T-C product. The pyrimidine dimers appear to have a range of influence that extends 1 to 3 base-pairs along the DNA molecule. These effects provide clues to the way DNA damage from mutagens and carcinogens can interfere with specific enzyme-DNA interactions. (Author abstract) (21 Refs)

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